UNIVERSITY
OF MICHIGAN
JUN 6 - 1956
LIBRARY

II. VOL. 18 NO. 6

TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

April 1956

THE NEW YORK ACADEMY OF SCIENCES

(Founded in 1817)

COUNCIL, 1956

President
WALTER S. ROOT

President-Elect
ROSS F. NIGRELLI

Vice-Presidents
EDWARD J. KEMPF BORIS PREGEL

Recording Secretary
CHARLES W. MUSHETT

Corresponding Secretary
FREDERICK C. NACHOD

Treasurer
RICHARD O. ROBLIN

Elected Councilors

1954-1956
JOHN M. CONVERSE B. M. DUGGAR
RANDOLPH T. MAJOR ABRAHAM SLAVIN

1955-1957
M. J. KOPAC LLOYD C. MILLER
C. P. RHOADS ELMER L. SEVERINGHAUS

1956-1958
DONALD B. KEYES CHARLES D. MARPLE
WARREN O. NELSON FREDERICK Y. WISELOGLE

Finance Committee
HARDEN F. TAYLOR, *Chairman*
GORDON Y. BILLARD ROBERT F. LIGHT

Executive Director
EUNICE THOMAS MINER

SECTION OF GEOLOGY AND MINERALOGY
M. HALL TAYLOR, *Chairman* ANASTASIA VAN BURKALOW, *Secretary*

SECTION OF BIOLOGY
HILARY KOPROWSKI, *Chairman* DANIEL LUDWIG, *Secretary*

DIVISION OF MYCOLOGY
JOHN B. ROUTIEN, *Chairman* KARL MARAMOROSCH, *Secretary*

SECTION OF PSYCHOLOGY
ALBERTA S. GILINSKY, *Chairman* RALPH F. HEFFERLINE, *Secretary*

SECTION OF ANTHROPOLOGY
JOHN L. LANDGRAF, *Chairman* HAROLD C. CONKLIN, *Secretary*

SECTION OF PHYSICS AND CHEMISTRY
FRANK COLLINS, *Chairman* ROBERT N. BOYD, *Secretary*

SECTION OF OCEANOGRAPHY AND METEOROLOGY
JEROME SPAR, *Chairman* EDWIN L. FISHER, *Secretary*

SECTION OF MATHEMATICS AND ENGINEERING
NICHOLAS V. FEODOROFF, *Chairman* S. B. LITTAUER, *Secretary*

Past Presidents
WILLIAM K. GREGORY VICTOR K. LA MER
HORACE W. STUNKARD M. L. CROSSLEY
HARDEN F. TAYLOR JOHN TERAVE
M. L. TAINTER

The Sections and the Division hold meetings regularly, one evening each month, during the academic year, October to May, inclusive.
Two-day conferences are also held at irregular intervals. All meetings are held at the building of The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, New York.

SER. II, VOL. 18 *April 1956* NO. 6

TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

Editor in Chief
KENNETH T. MORSE

Contents

Pleistocene-Periglacial Frost-Thaw Phenomena in New Jersey. *By* PETER E. WOLFE 507
The Microidentification of Organic Compounds, with Particular Application to Biological Systems. *By* NICHOLAS D. CHERONIS 516
Factors Influencing Growth in Protozoa:
I. Factors Influencing Abnormal Growth in Suctorian Protozoa. *By* FRANCIS J. STERBENZ AND DANIEL M. LILLY 522
II. Chemical Growth Supplements Promoting Growth in Carnivorous Ciliates. *By* DANIEL M. LILLY AND WILLIAM H. CEVALLOS 531
High-Speed Photography and the Natural Sciences. *By* JOHN H. WADDELL. 540
Symptom Mechanisms in Psychiatric Patients. *By* ROBERT B. MALMO..... 545
Studies on the Relationship Between Chemical Structure and Biological Activity of the *s*-Triazines. *By* H. P. BURCHFIELD..... 550
Ten Months of Operational Numerical Weather Prediction. *By* PHILLIP D. THOMPSON 555
The Antecedents of Zuni Culture. *By* RICHARD B. WOODBURY 557
Conferences Held..... 565
New Members 573

Copyright, 1956, by The New York Academy of Sciences

TRANSACTIONS of The New York Academy of Sciences, Series II, Volume 18, No. 6, April 1956.

Entered as second-class matter December 28, 1954, at the post office at New York, N. Y., under the act of August 24, 1912. This publication is distributed to members and is published monthly from November to June, inclusive, by The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, N. Y.

Executive Director: EUNICE THOMAS MINER

SECTION OF GEOLOGY AND MINERALOGY

PLEISTOCENE-PERIGLACIAL FROST-THAW PHENOMENA IN NEW JERSEY*

By Peter E. Wolfe

Rutgers University, New Brunswick, N. J.

Periglacial frost-thaw phenomena are of widespread occurrence south of the Wisconsin terminal moraine in New Jersey. Frost-thaw features include festoons, involutions, frost-wedge fillings and thermokarst lakes and depressions. The presence of faceted pebbles and widespread loess deposits likewise indicates that wind erosion and deposition was extensive in the periglacial zone.

The frost-thaw features occur on all Coastal Plain formations (Cretaceous and Tertiary) as well as on the Cape May gravels which are correlated with the pre-Wisconsin (Sangamon) interglacial stage. The lack of involutions in the horizons of the modern soil and the presence of cold-climatic peat flora (fir and spruce) indicate that the congeliturbate structures were formed prior to the present warm climatic period. The origin may therefore be dated as Wisconsin in time.

Location and Geologic Setting

The area in which the frost-thaw features were studied is located on the New Jersey Coastal Plain southeast of New Brunswick and Trenton, N.J., Philadelphia, Pa., and Wilmington, Del. (FIGURE 1). The Wisconsin terminal moraine occurs five miles north of New Brunswick; hence the Coastal Plain was subjected to a climate similar to that of other areas bordering a continental ice sheet. This climate is commonly referred to as a periglacial climate and is characterized by strong winds and extensive frost action.

The surface topography of the New Jersey Coastal Plain, in which the studies were carried out, is a low dissected plain that rises gradually from sea level at the coast to approximately 200 feet in the landward portion. Here and there, hills reaching elevations of 300 to 400 feet rise above the general surface level. Throughout the greater portion of the area, the relief is low, and the streams flow in open valleys that lie at only slightly lower levels than the broad, flat divides.

Lithologically, the Coastal Plain is made up of alternating beds of sand, silt, clay, greensand (glauconite) marl, lime marl, gravel, and other slightly consolidated rocks of the Cretaceous, Tertiary, and Quaternary ages. The beds dip gently (25 to 60 feet per mile) southeastward toward the Atlantic Ocean. Owing to the southeastward dip, the outcrops of the various formations trend northeast-southwest in fairly

*This paper was presented at a meeting of the Section on March 5, 1956.

regular bands, but since the dip of the strata is slight compared with the irregularities of the present surface, the outcrop bands curve with the topography. The total aggregate thickness of the Cretaceous and Tertiary formations varies from a few hundred feet along the outcrop margins to more than 1,500 feet along the coast. Overlying the Cretaceous and Tertiary formations are the Bridgeton (0 to 30 feet), the Pensauken (0 to 60 feet), and Cape May (0 to 30 feet) formations, which are of the Quaternary Age. These are interglacial gravels that are remnants of former extensive fluviatile deposits.

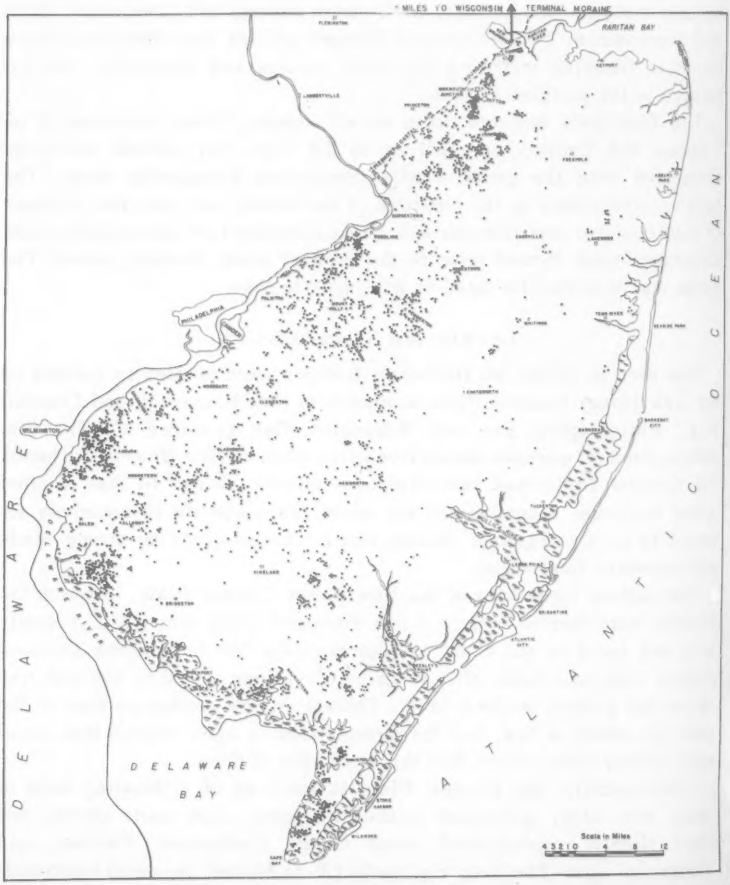


FIGURE 1. Basins with interior drainage on the New Jersey Coastal Plain. Legend: Each dot (·) represents a basin or group of basins as determined by aerial photographs.

Frost-Thaw Features

During the construction of the New Jersey Turnpike, numerous sections were exposed in the excavation operations. These sections, as well as numerous exposures in gravel pits, showed extensive evidence of frost action, including frost-wedge fillings, festoons, involutions, and other frost structures. A few of the localities studied are described below.

In the Dallenbach gravel pits, 1.5 miles northeast of Dayton, a variety of frost structures is found. These materials consist of several feet of clay overlaid by 6 to 8 feet of well-sorted and stratified sand of the Raritan formation. This, in turn, is overlaid by 4 to 10 feet of Pensauken gravel. With the exception of the upper 2 feet of the soil, these materials have been strongly involuted to a depth of 4 to 10 feet. Frost-heaved features similar to those in the Dallenbach pits are also found in the Herbert sand and gravel pits on the New Jersey Turnpike near Milltown. The geological materials are essentially the same as those in the Dallenbach pits, and the depth of disturbance is approximately the same. In the John Oris pits, one mile east of Roebling, involuted structures are well developed to a depth of 8 to 10 feet. The material in this pit differs from those already described in that it consists only of stratified silt and sand of the Cape May formation. The section includes 4 to 5 feet of undisturbed coarse and fine sand overlaid by 2 to 3 feet of strongly involuted silt and very fine sand. This, in turn, is overlaid by 5 to 7 feet of involuted fine sand, grading upward into the undisturbed soil. Similar congeliturbate structures are found in the Spilatore pits near Monmouth Junction, the Russell Ward pits near Palmyra, and many other areas of the Coastal Plain.

In all exposures studied, the congeliturbate structures were found to extend to depths varying from 3 to 10 feet. This depth of disturbance indicates that frost action penetrated to far greater depths than is possible under present climatic conditions. The undisturbed character of the upper soil horizons likewise indicates that the involuted structures are not being formed at present. They are readily explainable, however, as the result of frost action under a previously existing cold climate. Surficial structures of this kind, forming in arctic regions today, have been described by numerous authors (see Taber, 1945, and Hopkins, 1949).

Thermokarst Basins and Lakes

The thermokarst basins which occur in the New Jersey Coastal Plain are shallow depressions with a relief of less than 20 feet. They vary in size from a few hundred square feet to a square mile. Most of them are intermediate in size between these two extremes. Some of the depressions are oval or elliptical in shape. However, the majority are irregular in outline and without orientation.

There is a wide range of drainage conditions associated with different

basins. These vary from well-drained depressions, through those that are poorly drained and blanketed with peat and aquatic vegetation, to those that impound water as lakes. The well-drained depressions are usually associated with the sandy formations, while the poorly drained basins occur on clay and marl formations or sandy and gravelly areas underlaid with clay.

FIGURE 1 shows the geographic distribution of basins in New Jersey. This map was prepared from a study of aerial photographs supplemented by ground reconaissance. Differences in soil and vegetation, which in turn are expressions of drainage differences, were used in mapping the ponds and poorly drained depressions. The dark-colored areas in the aerial photograph (FIGURE 2) show the distribution of some of the typical poorly drained basins found near Cold Spring on the Cape May Peninsula, and similar depressions are of widespread occurrence elsewhere on the coastal Plain. Because of the uniformity of drainage and lack of soil and vegetation color differences in the well-drained basin areas, boundaries were delineated through stereoscopic studies. This method was particularly helpful in cultivated fields but was of less value in the forested areas, where the low basin relief was obscured by the trees. As a result, this map is incomplete, especially in the eastern sector, which is under forest cover.

The basins occur on all lithologic types, ranging from the heavy Cretaceous and Tertiary clays and marls to the light Tertiary sands and interglacial gravels. The greater concentration of poorly drained depressions in the western sector is a result of the higher clay content of the associated formations, particularly the interglacial gravels and Cretaceous and Tertiary clay and marl formations, and of the fact that they are in cultivated areas and more easily discernible on photographs.

Origin of Basins

Meteorite-scar hypothesis. Because of the previous work that has been done on the Carolina "bays" by Prouty (1952) and others and the fact that the origin of the bays has been attributed to falling meteorites, a magnetometer survey was carried out over several of the larger New Jersey basins. Thirty-seven stations covering an area of approximately two square miles were made, using an Askania vertical field balance. The magnetic anomalies obtained were small (less than 200 *y*) and were unrelated to the depressions. In the case of the Carolina bays, a 500 to 1,000 *y* variation is common, and these occur as spot magnetic highs. From the results obtained in this survey, there was no justification for attributing a meteoritic origin for the New Jersey basins. The facts that no meteorite fragments were found on the surface; that the basins lack the characteristic oval shape and rims of the Carolina bays; that they are without orientation; and that they are confined to unconsolidated forma-

tions would further point to the conclusion that they are not of meteoritic origin.

Solution depressions. The association of numerous basins with areas of soluble rock, particularly the Vincentown lime marl, led to the early belief that they might be solution depressions. Auger borings carried out on several basins showed that the relief of the basins was not due to the solution of the lime marl but to the distribution of the overlying insoluble quartz sand. FIGURE 3, a profile cross section made from auger borings along the line AB of one of the basins in the Vincentown area, shows the nearly Horizontal Vincentown lime marl overlaid by the Cape May sand and gravel. The widespread occurrence of depressions on other insoluble formations would further disprove the solution theory.

Frost-thaw basins. Taber (1930) has shown that frost heaving and frost thrusting take place in a thin, periodically thawed layer overlying perennially frozen ground. Frost turbulence results from a volume increase in the soil due to the segregation of masses of clear ice during freezing. When the soils are subjected to subfreezing temperatures in the presence of abundant moisture, they absorb a quantity of water greatly in excess of the natural porosity of the unfrozen material. The excess of water is present as segregations of clear ice, which commonly comprise 50 to 80 per cent of the volume of the unfrozen material. If clear ice wedges and lenses formed in equal abundance in all the materials at the surface, frost heaving would consist of a uniform dilation of the entire area. The surface of arctic regions is characterized, however, by numerous irregularities, including the character of the vegetation cover, the thickness of the peat and mineral soil, the quantity of moisture present, and the amount of snow cover during the critical autumn freezing period. Any one or any combination of these irregularities results in large local differences in the intensity of the frost heaving. In general, the wetter areas distend more than the drier areas because a larger supply of water is available for the formation of clear ice lenses. Likewise, ice lenses form more readily in silty soils than in peat, because water is drawn less readily through the large openings in the peat. Clear ice forms most abundantly in material that freezes first, because water is drawn laterally to the growing ice lenses in the freezing soil from the adjoining unfrozen areas. Thus, areas of bare soil or areas covered with a thin mantle of peat freeze rapidly and expand at the expense of the areas covered with dense vegetation. Vegetation also plays an important role in the distribution of the early snow cover, which in turn governs frost heaving during the critical autumn freezing period. Snowdrifts accumulate in areas covered with dense vegetation, while the bare soil areas are blown free of snow. As a result, the snow-covered sectors are insulated, while freezing temperatures penetrate rapidly into the snow-free areas and intense dilation takes place. Vegetation also plays an important role in the thawing of perennially frozen ground during the summer months. Hopkins (1949) has

FIGURE 2. Aerial photograph showing thermokarst basins on the Cape May Peninsula, N. J. Scale 1 inch to 4 miles.

shown that vertical ice wedges from 3 to 6 feet wide extend from the top of the perennially frozen layer to depths exceeding 10 feet. During the summer, thawing temperatures penetrate to depths ranging from 1 to 10 feet, depending upon the vegetal cover. Areas covered with a dense mat of vegetation are insulated, so that summer thaw penetrates only 1 to 3 feet. However, in areas of bare soil, the seasonal thaw extends to depths of 3 to 10 feet. Areas of deep thaw are initiated beneath frost boils and over buried ice wedges and where the vegetation cover is disrupted. The melting of the clear ice in local areas of deep thaw results in subsidence of the ground surface. Once formed, the areas are self-perpetuating because the resulting depressions hold pools of water beneath which thaw is accelerated and wave erosion and bank caving cause further enlargement. Peat-forming vegetation sometimes fills the lakes and, in other cases, the lakes are drained to form low, undulating flats surrounded by low ridges. The surface of arctic regions, as a result, consists of many depressions, peat-filled basins, and lakes, all of which are similar in many respects to those found in New Jersey.

The presence of deep frost-wedge fillings, involutions, and festoons in association with the New Jersey basins indicates that the area was subjected to intensive frost action under a climate more rigorous than that of the present time. Cold climatic conditions probably existed in the

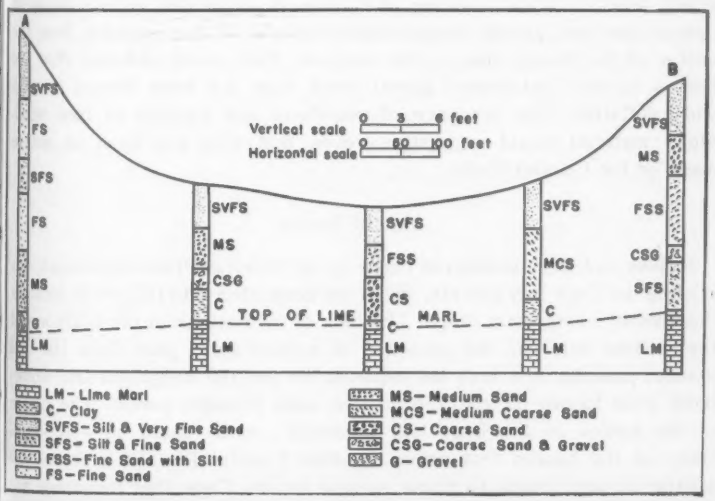


FIGURE 3. Profile of a thermokarst basin at Vincentown, N. J.

periglacial zone when the Ice Sheet stood just north of the Coastal Plain in Pleistocene time, and the development of these frost structures conceivably took place at this time. The differential freezing and thawing of perennially frozen ground during this period likewise would have produced the depressions similar to the thaw basins described by Hopkins (1949) which are being formed in arctic regions today.

Since the formation of the basins, many of the ponds and poorly drained areas have been covered with peat, ranging up to 10 feet in thickness. Preliminary studies of the pollen in these deposits made by Niering (personal communication, 1952) indicate that cold-climatic flora *Picea mariana, P. glauca, Tsuga,* and *Abies,* which were abundant in the peat bogs in the Pleistocene, have long since vanished from the pine barren area. Such data would further suggest the development of the basins prior to the present warm climatic period.

Wind action. Wind action has frequently been associated with frost action in the periglacial zone. Denny (1936) describes involutions in southern Connecticut associated with fine-grained wind-blown material containing ventifacts; and Selzer (1936) discusses ice-wedge structures formed concurrently with the deposition of loess. The widespread occurrence of ventifacts and deposits of loess in New Jersey indicates that the area was subjected to strong wind action. Patches of fine-grained wind-blown material have been found overlying the basins in several areas, and local dune topography is common on the sandy formations. Data collected from auger borings and pit studies in the frost-heaved gravel areas indicate, however, that there is no greater concentration of coarse sand and gravel or appreciable removal of fine material from the center of the basins than at the margins. This would indicate that the basins in the frost-heaved gravel areas have not been formed through wind deflation. The presence of ventifacts and patches of fine wind-blown material would suggest, however, that wind has been an active agent on the Coastal Plain.

Age of Basins

Basins and frost structures occur on all Coastal Plain formations, including the Cape May gravels, which are correlated with the pre-Wisconsin (Sangamon) interglacial stage. The lack of involutions in the horizons of the modern soil and the presence of cold-climatic peat flora (fir and spruce) indicate that both the depressions and the congeliturbate structures were formed prior to the present warm climatic period. The origin of the basins on the Cape May formation can be dated as Wisconsin. Many of the basins that occur on other Coastal Plain formations are similar in appearance to those present on the Cape May formation and are assumed to be of the same age. Evidence for earlier periods of origin (Illinoian and Kansan, which are recorded in New Jersey) are lacking.

However, if frost action occurred during one period, it is reasonable to believe that it occurred during earlier periods as well.

References

DENNY, C. S. 1936. Periglacial phenomena in southern Connecticut. Am. J. Sci. **37**: 322-342.

HOPKINS, D. M. 1949. Thaw lakes and thaw sinks in the Imuruk Lake area, Seward Peninsula, Alaska. J. Geol. **57**: 119-131.

NEIRING, W. (1952) Personal communication. Dept. Botany. Rutgers Univ. New Brunswick, N. J.

POTZEGER, J. E. 1945. The pine barrens of New Jersey, a refugium during Pleistocene Times. Butler Univ. Bot. Studies. **7**: 1-15.

PROUTY, W. F. 1952. The Carolina bays and their origin. Bull. Geol. Soc. Amer. **63**: 167-224.

SELZER, G. 1936. Diluviale Loesskerle und Loesskeilnetze aus der Umgebung Gottingens. Geol. Rundschau. **27**: 275-293.

TABER, S. 1930. The mechanics of frost heaving. J. Geol. **38**: 303-317.

TABER, S. 1943. Perennially frozen ground in Alaska; its origin and history. Bull. Geol. Soc. America. **54**: 1433-1548.

WOLFE, P. E. 1953. Periglacial frost-thaw basins in New Jersey. J. Geol. **61**(2): 133-141.

SECTION OF PHYSICS AND CHEMISTRY

THE MICROIDENTIFICATION OF ORGANIC COMPOUNDS, WITH PARTICULAR APPLICATION TO BIOLOGICAL SYSTEMS*

By Nicholas D. Cheronis

Department of Chemistry, Brooklyn College, Brooklyn, N. Y.

The classic approach to the identification of organic compounds is based on methods and procedures by which it is possible to prove that an unknown substance *A* is identical with a known substance *B*. A rigorous proof[1] would require establishing, first, that *A* and *B* have substantially the same physical properties; and, second, that *A* and *B* exhibit similar chemical properties. In practice, the physical constants that are compared are usually solubilities,[2] melting points, boiling points, and refractive indices. Other constants that are employed at times are the infrared and various other types of spectra,[3,4] R_f values of chromatograms, distribution constants between two immiscible solvents, and crystallographic properties.[5,6] The similarity in chemical behavior is usually established by comparison of functional group reactions and production of the same derivative *D* when *A* and *B* are reacted separately with the same reagent *R*. The comparison of functional group reactions between *A* and *B* is usually accomplished by chemical tests for the production of similar colored complexes, or precipitates, or gaseous substances. The proof that derivatives from *A* and *B* are similar is usually established by comparison of melting points, or by mixed fusion.[5,6]

In the systematic schemes[7-19] for the identification of organic compounds, the first step is to isolate a relatively pure compound by means of fractionation procedures. In establishing the purity of the isolated component, one or two physical constants are determined. In the next step, the unknown is classified into a large division of compounds on the basis of the elements present, solubility in selected solvents and/or reaction to indicators.[20] This is followed by restriction of the classification of the unknown to a single class of organic compounds by means of functional group tests. It is possible now, by correlation of the experimental data and a search of the literature, to find a known compound *B* that bears resemblance to the unknown *A*. Thus a tentative assumption is made that the unknown compound *A* is identical with the compound *B* listed in the literature. The final and conclusive step leading to the confirmation of the *tentative* proof is the preparation of suitable derivatives. The unknown is reacted with a number of reagents, and two or more derivatives are prepared. If the melting points of the derivatives are substantially the same as those listed in the literature for the same derivatives of the compound tentatively assumed to be identical with the

*This paper was presented at a meeting of the Section on March 6, 1956.

unknown, the proof can be made conclusive by mixed melting points or, better, by fusion techniques.[5,6]

Proofs of identity based on the comparison of one or two physical constants, or both a physical constant and a functional group test, are common not only in routine work but also in many research reports as to the presence of organic compounds in mixtures of biological origin. In routine work, the presence of an expected compound or the absence of a substance is frequently concluded from one or two tests that are based upon the formation of a precipitate or a colored complex. Although such practice is admissible in routine work, the identification of an unknown should be based on more than similarity of one or two constants. The presence of a particular compound in microgram quantities in a biological fluid on the basis of R_f values and location of spots on a paper chromatogram should be regarded as *tentative*. For example, of approximately 70 organic compounds including 1,2-benzpyrene and 3,4-benzpyrene that have been reported[21] in "mainstream" cigarette smoke, most of the evidence for the presence of these compounds is based on infrared and ultraviolet spectra and chromatographic data. The conflicting reports as to the presence of benzpyrenes and other carcinogens in cigarette smoke are due to the fact that the claims are not founded upon *rigorous* proofs. For a rigorous proof it would be necessary to isolate milligram or microgram quantities of these carcinogens and corroborate their identity by derivatization.

The chief problems in the identification of organic compounds encountered in biological systems are essentially those that involve the separation of small quantities of the individual components of a mixture in a state of purity sufficient to permit systematic characterization. Once relatively pure substances have been isolated, either in milligram or microgram quantities, procedures have to be outlined[1] to obtain rigorous identification. Though great advances have been made in the fractionation of small quantities of organic compounds by the introduction of column adsorption, paper chromatography, and ion exchangers, these techniques are almost entirely empirical and, as yet, no systematic approach has been made to the fractionation of mixtures containing milligram or microgram quantities of several organic compounds. For example, assume that an extract from plant tissues contains milligram quantities of several monoprotic and diprotic acids, alcohols, esters, aldehydes, ketones, amino acids, sugars, and salts of heterocyclic bases. At present there is no systematic procedure by which separate samples of the extract may be fractionated so as to obtain first acids, alcohols, esters, aldehydes, ketones, and amino compounds, and then each group refractionated into the individual homologs. Notwithstanding the prodigious amount of work that has been done on the fractionation of small quantities of amino acids by means of paper chromatography, reports that

include rigorous identification are few. An outstanding example of such an approach is the work of Dent and his co-workers[22] in their proof that the T-spot found in the chromatograms of normal urine is due to the presence of β-aminoisobutyric acid. The component causing the T-spot was concentrated, then fractionated by means of ion exchangers until a crystalline substance melting at 183 to 184° C. was obtained. This substance then was shown by stepwise and systematic analysis to be identical with DL-β-aminoisobutyric acid.

During the past few years, work carried on in the author's laboratory has been concerned with the problem of developing a systematic procedure for the separation of small amounts of organic compounds in synthetic mixtures, and later of trace components that occur in plant-tissue extracts such as fruit juices.[23] The use of ion exchangers for the separation of a number of carboxylic and amino acids from maple syrup, fruit juices, and other plant-tissue extracts has been reported by Bryant and Overell,[24] Hass and Stadtman,[25] Wilker,[26] and Mills.[27] The use of ion exchangers for the separation of carbonyl compounds from synthetic mixtures has been reported by Gabrielson and Samuelson.[28] Rice, Keller, and Kirchner[29] reported the separation and identification of small quantities of carbonyl compounds and alcohols from orange juice by separation of the 2,4-dinitrophenylhydrazones and 3,5-dinitrobenzoates, respectively, followed by fractionation by means of paper chromatography.

The approach that is being currently investigated and through which some progress has been made employs a combination of physical processes that involve steam distillation, liquid-liquid extraction, and ion exchangers to separate the mixture so as to obtain fractions containing one or two groups of organic compounds, which are then further resolved into individual groups and components by column-adsorptive and paper-chromatographic procedures. In a tentative scheme, the plant extract is first steam-distilled to give a volatile fraction (V-I) containing the volatile monoprotic acids C_1-C_6, most of the alcohols and carbonyl compounds, leaving a residue (R-I) that contains the higher monoprotic acids, most polyprotic acids, all amino acids, polyhydroxy compounds, sugars, and salts of heterocyclic bases. Fraction V-I is passed through an anion exchanger activated by sodium bicarbonate that retains by sorption the monoprotic acids and the phenolic hydroxy and carbonyl compounds (fraction V-II), leaving in the effluent (fraction V-III) most of the carbonyl and hydroxy compounds. Fraction V-III is then passed through an anion exchanger activated with sodium bisulfite that retains by sorption the carbonyl compounds (fraction V-IV) and leaves the alcohols and esters in the effluent (fraction V-V).

The fraction (V-II) containing the volatile monoprotic acids is further fractionated after elution by dissolving it in cyclohexane or benzene and passing it through a column containing silicic acid buffered at various

pH's. The various fractions are derivatized as 2,4-dinitrophenylhydrazides[30] and further fractionated by paper chromatography. Fraction V-IV, which contains the carbonyl compounds, is fractionated further by dissolving in benzene and passing through a column containing silicic acid and alumina.[31] By this procedure it is possible to effect a separation into aliphatic ketones (R_f:0.4), aliphatic aldehydes (R_f:0.5), and aromatic aldehydes (R_f:0.75).[31] The various zones are identified by means of streak reagents.[32a,b] The various fractions of the carbonyl compounds are further refractionated by conversion to 2,4-dinitrophenylhydrazones followed by paper or column chromatography. Finally, fraction V-V, which contains the alcohols and esters, is further refractionated by passing it through a column containing silicic acid. The alcohols are tentatively identified by paper chromatography of their 3,5-dinitrobenzoates. No satisfactory procedure has been developed for the tentative identification of milligram quantities of esters.

The residue (R-I) from steam distillation containing the higher monoprotic acids, most of the polyprotic acids, and all of the amino acids, polyhydroxy compounds, heterocyclic bases, and sugars is passed through a cation exchanger that retains the amino acids, heterocyclic bases, and inorganic ions (fraction R-II). The effluent (fraction R-III) is passed through an anion exchanger activated with sodium bicarbonate that retains (fraction R-IV) the higher monoprotic acids, the polyprotic acids, and the phenolic hydroxy and carbonyl compounds. The effluent (fraction R-V) contains the sugars. By elution of the various fractions and refractionation through adsorbents in columns or paper chromatograms, it is possible to identify tentatively most of the components present in the mixture. For example, from the centrifuged juice of watermelon pulp, four monoprotic acids, three carbonyl compounds, two alcohols, and two amino acids have been identified,[23] while about 10 minor components remain to be identified.

After the minor components of a mixture have been separated into relatively pure components, procedures have been described[1,5,8,33] through which the systematic characterization of each pure component is feasible even when the quantity of substance available is 1 mg. or less. The main problem in such characterizations consists in preparing suitable derivatives from milligram or microgram quantities and then purifying the derivatives and determining their melting points. In the cases of alcohols, carbonyl compounds, and carboxylic acids, exact methods[34,35] have been developed by which derivatives may be prepared from 1 mg. or less of a substance and the crude product purified by fractional vacuum sublimation. Thus it is possible to react 1 mg. or less of an alcohol with α-naphthylisocyanate and then fractionate the resulting crude urethan in an appropriate vacuum microsublimator so as to obtain three fractions of crystalline material. The middle fraction consists

of the pure urethan. Of several alcohols derivatized in this manner, the middle fraction gave values for melting points that either corresponded to the exact value recorded in the literature or deviated by 0.5 to 1° C.

In connection with the derivatization of microgram quantities of organic substances, attention should be paid to the concentration of the reactants. The usual procedure of adding a few drops of solvent to a few micrograms of substance and the derivatizing reagent results in concentrations of about 10^{-3} to 10^{-4}M. Very little information exists in the literature as to the rates of organic reactions at concentrations below 10^{-1}M. Investigations of the rates of reactions of several aliphatic and aromatic carbonyl compounds with 2,4-dinitrophenylhydrazine at concentrations of from 10^{-1} to 10^{-4}M indicate[36] that with the lower homologs (acetaldehyde and acetone) that are expected to react at appreciable rates, the reaction is complete within a few minutes at a concentration of 10^{-1}M. At concentrations of 5×10^{-2}M, the rate reaches a plateau at about 70 per cent completion within 15 minutes and, at 5×10^{-3}M, the rate is so slow that even after several days the reaction does not proceed above 50 per cent. The rates of 2,4-dinitrophenylhydrazone formation cannot be predicted on the basis of the current reaction mechanisms. The factors that appear to be important are solubility and nature of the radical *R* attached to the carbonyl group. Thus *p*-anisaldehyde gives a 2,4-dinitrophenylhydrazone at concentrations of 5×10^{-4}M. Generally, however, it appears that in order that organic reactions may take place under operational conditions, the concentration of the reactants should be kept as near to 10^{-1}M as possible.

The problem of derivatizing microgram quantities of a mixture of several homologs and then identifying them by means of their infrared or X-ray spectra is under investigation. Under certain conditions, this would provide a means for a rigorous identification of minor components of biological systems without the present difficulties of fractionating a mixture containing milligram or microgram quantities of several homologs in order to obtain relatively pure compounds.

References

1. CHERONIS, N. D. 1954. Micro and semimicro method. *In* A. Weissberger, Ed. Technique of Organic Chemistry. **6**: 415-417. Interscience Publishers. New York, N. Y.
2. KAMM, O. 1922. Qualitative Organic Analysis. John Wiley and Sons. New York, N. Y.
3. RANDALL, H. M., N. FUSON, R. G. FOWLER & J. R. DANGLE. 1949. Infrared Determinations of Organic Structures. D. Van Nostrand Co. New York, N. Y.
4. DUNCAN, A. B. F. *et al.* 1956. Chemical Applications of Spectroscopy in A. Weissberger, Ed. Technique of Organic Chemistry. **9**: Interscience Publishers. New York, N. Y.

5. KOFLER, L. & A. KOFLER. 1954. Thermo-Mikro-Methoden. Verlag Chemie. Weinheim/Bergstrasse, Germany.
6. MCCRONE, W. C. 1956. Fusion Methods of Analysis. Interscience Publishers. New York, N. Y.
7. CAMPBELL, N. 1939. Qualitative Organic Analysis. John Wiley and Sons, New York, N. Y.
8. CHERONIS, N. D. & J. B. ENTRIKIN. 1956. Semimicro Qualitative Organic Analysis. 2nd ed. Thomas Y. Crowell and Co. New York, N. Y.
9. CLARKE, H. T. 1926. Handbook of Organic Analysis, 4th ed. Edward Arnold and Co. London, England.
10. EKKERT, L. 1933. Erkennung organischer Verbindung im besonderen von Arzneimitteln. F. Enke. Stuttgart, Germany.
11. HUNTRESS, C. H. & S. P. MULLIKEN. 1941. Identification of Pure Organic Compounds. **1**: John Wiley and Sons, New York, N. Y.
12. MCELVAIN, S. N. 1945. Characterization of Organic Compounds. MacMillan and Co. New York, N. Y.
13. MEYER, H. J. L. 1933. Nachweis und Bestimmung organischer Verbindungen. J. Springer. Berlin, Germany.
14. MIDDLETON, H. 1943. Systematic Qualitative Organic Analysis. 2nd ed. Edward Arnold and Co. London, England.
15. ROSENTHALER, L. 1923. Der Nachweis organischer Verbindungen. 2nd ed. F. Enke. Stuttgart, Germany.
16. SHRINER, R. L., R. C. FUSON & D. Y. CURTIN. 1956. The Systematic Identification of Organic Compounds. 4th ed. John Wiley and Sons. New York, N. Y.
17. SMITH, F. J. & E. JONES. 1953. A Scheme of Qualitative Organic Analysis. Blackie and Son. London, England.
18. STAUDINGER, S. 1925. Anleitung zur Organischen Qualitativen Analyse. J. Springer. Berlin, Germany.
19. VEIBEL, S. 1954. The Identification of Organic Compounds. 4th ed. G.E.C. Gad. Copenhagen, Denmark.
20. DAVIDSON, D. 1942. J. Chem. Educ. **19**: 221.
21. KOSAK, A. I. 1956. The Chemical Composition of Smoke. Symposium on Tobacco. Metropolitan and Long Island Section. Am. Chem. Soc. Feb. 24, 1956.
22. DENT, C. E. *et al.* Nature. **167**: 307.
23. CHERONIS, N. D. & S. ELEFTHERIOU. 1956. Microidentification of Trace Components in Fruit Juices. Symposium. Metropolitan Microchemical Society. April 1956. New York, N. Y.
24. BRYANT, F. & B. T. OVERELL. 1951. Nature. **167**: 361.
25. HASS, V. A. & E. R. STADTMAN. 1949. Ind. Eng. Chem. **41**: 983.
26. WILKER, W. O. 1951. J. Assoc. Offic. Agr. Chemists. **34**: 506.
27. MILLS, P. A. 1951. J. Assoc. Offic. Agr. Chemists. **34**: 506.
28. GABRIELSON, G., & O. SAMUELSON. 1950. Svensk. Kem. Tidskr. **62**: 214.
29. RICE, R. G., KELLER, G. J. & J. G. KIRCHNER. 1951. Anal. Chem. **23**: 194.
30. CHERONIS, N. D. & S. COHN. 1956. Mikrochim. Acta. **4**: 925.
31. CARLTON, J. K. & W. C. BRADBURY. 1955. Anal. Chem. **27**: 67.
32a. LE ROSEN, A. L., MORAVEK, R. T. & J. K. CARLTON. 1952. Anal. Chem. **24**: 1335.
32b. CARLTON, J. K. 1956. Personal Communication.
33. SCHNEIDER, F. 1946. Qualitative Organic Microanalysis. John Wiley and Sons. New York, N. Y.
34. CHERONIS, N. D. & A. VAVOULIS. 1952. Mikrochim. Acta. **38**: 428.
35. CHERONIS, N. D. 1954. Micro and Semimicro Methods. In Technique of Organic Chemistry. A. Weissberger, Ed. **6**: 479-491. Interscience Publishers. New York, N. Y.
36. CHERONIS, N. D., V. M. LEVEY & H. STEIN. 1956. Derivatization of Small Quantities of Organic Compounds. Symposium Metropolitan Microchemical Society. April 1956. New York, N. Y.

SECTION OF BIOLOGY

FACTORS INFLUENCING GROWTH IN PROTOZOA

I. FACTORS INFLUENCING ABNORMAL GROWTH IN SUCTORIAN PROTOZOA*

By Francis J. Sterbenz and Daniel M. Lilly

Department of Biology, St. John's University, Brooklyn, N. Y.

That suctorians might be a unique tool for biological research has been realized for many years; however, their full potential value has yet to be exploited. The primary problem is one of nutrition, or the controlled production of a useful number of organisms. This lack of sufficient numbers of organisms has hampered certain cytological investigations and has limited physiological studies. This report summarizes some recent experimental work concerning factors influencing the growth of suctorians.

The early investigations in this laboratory have been reported in the *Annals* by one of us[1] and will receive brief mention here only for the sake of continuity. In "wild" cultures contaminated with bacteria, yeasts, molds, and protozoa other than those noticed to be utilized as food, some species of suctorians seemed to thrive upon the ciliate *Tetrahymena pyriformis*. Declines, characterized by the production of abnormal forms with the simultaneous failure of normal reproduction, have been noted after a variable length of time. These declines have been attributed to the presence of unfavorable bacteria, pH changes, and other similar detrimental factors.[2,3,4] These observations have permitted the entertaining of the idea that an unlimited number of suctorians could be made available by means of two-member cultures.

Unfortunately, upon placing suctorians in monoxenic cultures with *T. pyriformis* in water, the nutritional inadequacy of the ciliate became evident. Abnormalities, such as those seen in declining "wild" cultures, predominated. It was soon discovered[5] that the addition of yeast extract to the water in which *Tokophrya infusionum* was grown limited the number of abnormal forms as long as small numbers of the food ciliate were made available. When large numbers of the ciliates were given, however, abnormal forms soon predominated and, if the excess food organisms were not promptly removed, the culture was soon completely destroyed. Rudzinska[6,7] reported similar findings and also observed that storage at low temperature (10° C.), combined with sparse feedings, greatly aided in the prevention of abnormal forms.[8,9,10,11]

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on March 12, 1956.

The investigations described in this paper were supported in part by a research grant-in-aid from the American Cancer Society, New York, N.Y., on the recommendation of the Committee on Growth of the National Research Council, Washington, D.C.

The beneficial effect of yeast extract was first investigated in this laboratory[1,12] to determine just which component was the factor active in the prevention of abnormal organisms. It was found that yeast nucleic acid could substitute for the whole extract and that, specifically, guanylic acid was the substance which had the greatest activity in the prevention of abnormal forms of *Tokophrya*.

In attempts to culture another suctorian species, *Podophrya collini*, it was observed that yeast and guanylic acid, rather than preventing, increased the percentage of abnormal forms.[13,14] The first substance that tended to prevent abnormalities was the guanine antagonist, 8-azaguanine. Subsequently it was found that the substituted purines theobromine, theophylline, and caffeine were also similarly effective.

During the production of a phase-contrast film record of the effect of substituted purines upon two species of suctorians[15] it was noted that the organisms became increasingly opaque as feeding progressed, so that little if any internal structure could be seen. Thus it was not possible to observe nuclear phenomena occurring before and during reproduction. Since facilities for ultraviolet microscopy were available at the Brookhaven National Laboratory, Upton, N.Y., preliminary studies were conducted in association with M. J. Moses[16] upon one of the suctorian species, *P. collini*. Ultraviolet photomicrographs were made of organisms in different nutritional conditions to determine if there was a demonstrable change in the nucleic acid content. Correlated with the photographs, appropriate cytochemical tests for nucleic acids and proteins were also employed. Well-fed suctorians and those that had developed into abnormal forms were quite opaque and granular in appearance with ordinary illumination. In the ultraviolet photographs these well-fed and abnormal forms were so opaque that no cytological details could be distinguished. Suctorians that had not been fed for several days became quite transparent in visible light. Nevertheless, in ultraviolet photographs the starved forms were just as opaque as the well-fed and abnormal forms. Apparently the nucleic acid content of these organisms was always much higher than that of *Tetrahymena* since, in the photographs, the suctorians were much more opaque than the ciliates.

The cytochemical tests revealed that most of the nucleic acids in the suctorians were in the form of ribonucleic acid in the cytoplasm. This was readily removed by digestion with ribonuclease. The desoxyribonucleic acid, as indicated by the Feulgen reaction, was mainly confined to the macronucleus and the micronuclei. In many organisms, however, smaller Feulgen positive bodies were visible; the exact nature of these has not yet been determined. It is possible that they may represent ingested nuclear material from the *Tetrahymena*.

By treating *Podophrya* with guanylic acid, large numbers of abnormal forms were produced. No significant increase in desoxyribonucleic acid

TABLE 1

THE EFFECT OF TEMPERATURE UPON THE RESPONSE OF *TOKOPHRYA INFUSIONUM* TO ISOBARBITURIC ACID

Additions	*19° C.*	*26° C.*
Isobarbituric acid 250 μg./ml.	15% abnormal forms	50% abnormal forms
No additions	0% abnormal forms	24% abnormal forms
Uracil 10 μg./ml.	0% abnormal forms	0% abnormal forms

Culture fluid consisted of 20 per cent Medium A of Kidder and Dewey, less Tween 80, dextrose, purines, and pyrimidines, with 90 μg./ml. guanylic acid added.

was apparent in the development of the abnormal forms, but there was evidently a considerable increase in ribonucleic acid and protein. There was also an apparent difference in digestibility with ribonuclease when abnormal forms were compared with normal suctorians.

The proteins of *Podophrya* also present some interesting problems. The tentacles and stalk of this suctorian show a high content of protein with the fast green technique. The unusual structural characteristics of these organelles may involve special types of proteins. Electron microscope studies by Rudzinska and Porter[17] on *Tokophrya infusionum* have shown that the food vacuoles contained mitochondria and lipids from the food ciliate. The lipid content of the vacuoles is probably the reason for the loss of transparency of the suctorians when they begin to feed on ciliates.

TABLE 2

THE EFFECT OF TEMPERATURE UPON THE RESPONSE OF *TOKOPHRYA INFUSIONUM* TO 8-AZAGUANINE AND FLAVOTIN

Additions	*19° C.*	*26° C.*
8-azaguanine 1 μg./ml.	0% abnormal forms	32% abnormal forms
8-azaguanine 1 μg./ml. plus flavotin 10 μg./ml.	0% abnormal forms	93% abnormal forms
Flavotin 10 μg./ml.	0% abnormal forms	33% abnormal forms
No additions	0% abnormal forms	3% abnormal forms

Culture fluid consisted of 20 per cent Medium A of Kidder and Dewey, less Tween 80, dextrose, purines, and pyrimidines, with 10 μg./ml. guanylic acid and 10 μg./ml. uracil added.

It was found that a modified Gram stain could be employed to stain the lipid-containing vacuoles in well-fed and abnormal forms.

During the time when this preliminary work was in progress, a special television camera sensitive in the ultraviolet became available for use with the ultraviolet microscope. It was then possible to observe the process of feeding more readily on the screen of the monitor. With the ultraviolet source used, however, an exposure of approximately 10 minutes was sufficient to cause cytolysis of the food ciliates. No visible changes were observed in the suctorians after much longer exposures; even organelles as delicate as the tentacles remained extended and unchanged in appearance. Later it was found that such exposed suctorians were not viable. The lack of cytolysis may involve peculiarities of the membranes of suctorians.

In all of the test-tube cultures studied, the suctorians grew either attached to the tube in a narrow ring just below the meniscus or floating on the surface of the culture fluid. Soon after the organisms were submerged, due to the addition of fluid with repeated small feedings, they transformed into abnormal forms. Thus only a limited number of normal reproducing forms were available in a culture at any stated time; this may be contrasted with *Tetrahymena*, in which thousands of organisms per ml. were not unusual. Various observations led to the belief that the primary reason the suctorians were so subject to variation and so narrowly distributed was not only because the young swimming forms were negatively geotropic but that both forms were very aerobic. This hypothesis has been tested by aerating cultures of *Podophrya*.[18]

The method used was to bubble sterile air through capillary tubes directly into growing cultures. The method proved to be effective as the organisms were well distributed throughout the test tube, with many now found deep in the tube near the capillary. The disadvantages of this method of culture consisted in the difficulty of equalizing the aeration in several cultures at one time and the inherent danger of bacterial contamination upon repeated feedings due to the nature of the apparatus.

A second method of aeration consisted of a modified roller tube as used in tissue culture. There were slightly greater numbers of organisms produced per feeding, as compared to stationary control cultures, but most of them were found as floaters due to the now greatly expanded surface area of the fluid. This prevented their easy observation for the estimation of the results of an experiment. The organisms that were attached to the wall of the tube were no longer periodically submerged when taken from the rotator, and they changed their morphology rapidly, tending to confuse results. Although this method did tend to promote reproduction in the early stages of the culture, there was always a large percentage of abnormal forms in slightly older cultures even when stationary controls had few. Thus it may be seen that these methods

would have prevented the accomplishment of the few qualitative experiments that have been done.

During the course of these experiments a striking temperature phenomenon was observed. The optimal temperature for the culture of these organisms was found to be between 18 and 20° C. Temperatures ranging from 1 to 3° C. and from 25 to 30° C. proved to be quite detrimental to the organisms under certain experimental conditions. TABLE 1 presents the effect of temperature upon the response of *Tokophrya infusionum* to the effects of isobarbituric acid. It should be noted that in the absence of uracil there was a considerable number of abnormal forms in the control cultures grown at 26° C., while there were none in those grown at 19° C., indicating the probable need for greater concentrations of uracil for normal reproduction at these higher temperatures. TABLE 2 presents the effect of temperature upon the response of *Tokophrya* to 8-azaguanine and flavotin, a riboflavin analog, separately and in combination. It may be readily seen that higher temperatures increased the sensitivity of the organisms to the detrimental effects of the antimetabolites. This was not because the suctorians were visibly inhibited by the cooler temperatures. On the contrary, there was a greater number or organisms, indicating that more reproduction occurred in the cultures grown at 19° C. than in those grown at 26° C. The best explanation for these results appears to be that the incorporation of the analogs was reduced under these conditions. This tends to confirm the results obtained by Kidder, Dewey, and Parks,[19] who observed that the inhibitory power of 8-azaguanine is greatly reduced for cultures of *Tetrahymena* in which any one of a number of essential metabolites is limited.

These observations of the effects of antimetabolites seemed to indicate, at one time, that the ratio of purine to pyrimidines was important, even though the results obtained were not always in accord with theoretical expectations. Further investigation has not supported this interpretation. In fact, some experiments have indicated that the effect of purines has no direct relationship to the effect of pyrimidines. The nonuniformity of response reported can now be attributed to the probability that suctorians do not obtain a significant amount of their nutrients without a food ciliate as intermediary.

When *Tokophrya infusionum* was exposed to an antimetabolite in the absence of *Tetrahymena,* no abnormal forms were produced. After the introduction of *Tetrahymena* simultaneously with an antimetabolite, the percentage of abnormal forms produced was roughly proportional to the concentration of the antimetabolite, but reversal by the corresponding metabolite was not always as expected by theory. When the *Tetrahymena* were grown in the presence of an antimetabolite before the suctorians were permitted to feed upon them, the percentage of abnormal forms was always greater than could be expected to be caused by low concentra-

tions. Evidently, then, the fault in the experiments conducted by adding the test substance and the food ciliate simultaneously was that the amount of antimetabolite active upon the suctorians depended upon the variable time the ciliates were exposed to the test substance as well as the number of ciliates eaten by a suctorian. Thus, if the suctorians in a particular tube were able to feed upon most of the ciliates sooner than those in another tube in the same series, the former would have a lower percentage of abnormal forms due to the lower concentration of the antimetabolite in the ciliates at the time they were eaten. In like manner, if the ciliates are pretreated with the antimetabolite, they concentrate it, and the percentage of abnormal suctorian forms produced becomes greater than could be theoretically expected. These results, using the guanine antagonist 8-azaguanine, are illustrated in TABLE 3.

The riboflavin analog flavotin has been reported to be inert in *Tetrahymena*, neither substituting for riboflavin nor antagonizing it, while being relatively active in producing abnormal forms of *Tokophrya*.[20] FIGURE 1 presents this data on *Tetrahymena*, and TABLE 4 illustrates that although the ciliate was not affected by flavotin, it apparently contained it and made it available to the suctorian.

When concentrations of either 8-azaguanine or flavotin were such that they did not affect the suctorians, a synergistic effect seems evident upon exposure to the two simultaneously. That this effect occurred at much lower levels when the food ciliate was pretreated is recorded in the data of TABLE 5.

TABLE 3

THE EFFECT OF PRETREATMENT OF *TETRAHYMENA PYRIFORMIS* UPON THE RESPONSE OF *TOKOPHRYA INFUSIONUM* TO 8-AZAGUANINE*

8-azaguanine	*Per cent abnormal forms Produced 26° C.*	
	Pretreated	*Not pretreated*
0.001 μg./ml.	42	
0.01 μg./ml.	84	52
0.1 μg./ml.	100	75
1.0 μg./ml.	100	89

Culture fluid consisted of 20 per cent Medium A of Kidder and Dewey, less Tween 80, dextrose, purines, and pyrimidines, with 10 μg./ml. guanylic acid and 10 μg./ml. uracil added.

*The importance of controlling the chronological age as well as the physiological state of the suctorians used as an inoculum is evident when one compares the results recorded above with those of TABLE 2. In the experiments of this table and those following, the reproduction within the stock cultures was synchronized so that only embryos were used as inoculum. In the previous experiments the method of controlling this process was not known, so adults of various ages as well as embryos were used.

Although our knowledge of the nutrition of other organisms in axenic culture is considerable, and the difficulties encountered when working with two-member cultures, especially in the interpretation of experimental

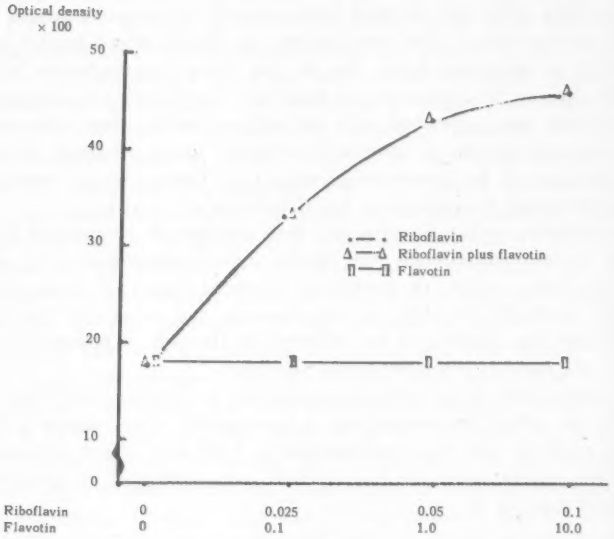


FIGURE 1. The ineffectiveness of flavotin as an antagonist or as a substitute for riboflavin in *Tetrahymena pyriformis*.

TABLE 4

THE RESPONSE OF *TOKOPHRYA INFUSIONUM* TO FLAVOTIN FOLLOWING THE PRETREATMENT OF *TETRAHYMENA PYRIFORMIS*

Flavotin	*Per cent abnormal forms produced 19° C.*	
	Pretreated	*Not pretreated*
0.01 μg./ml.	18	-
0.1 μg./ml.	85	0
1.0 μg./ml.	90	12
10.0 μg./ml.	100	18

Culture fluid consisted of 20 per cent Medium A of Kidder and Dewey, less Tween 80, dextrose, purines, and pyrimidines, with 10 μg./ml. guanylic acid and 10 μg./ml. uracil added.

TABLE 5

THE RESPONSE OF *TOKOPHRYA INFUSIONUM* TO COMBINATIONS OF 8-AZAGUANINE AND FLAVOTIN FOLLOWING THE PRETREATMENT OF *TETRAHYMENA PYRIFORMIS**

Antimetabolite		*Per cent abnormal forms produced 19° C.*	
8-azaguanine μg./ml.	*flavotin μg./ml.*	*Pretreated*	*Not pretreated*
0.01	0.1	82	8
0.01	1.0	100	25
0.01	10.0	100	35
0.1	0.1	100	18
0.1	1.0	100	40
0.1	10.0	100	66
1.0	0.1	100	38
1.0	1.0	100	63
1.0	10.0	100	73

Culture fluid consisted of 20 per cent Medium A of Kidder and Dewey, less Tween 80, dextrose, purines, and pyrimidines, with 10 μg./ml. guanylic acid and 10 μg./ml. uracil added.

*The percentages reported in this and the preceding tables are averages of at least two experiments containing a minimum of six duplicates. The absolute response to an antimetabolite in an experiment is constant only when precautions are observed to maintain a constant physiological condition in the inoculum.

data, is acknowledged, no solution to the problem of the axenic culture of these organisms has been found. It seems evident from our experimental observations that little, if any, nutrient is obtained through the pellicle of a suctorian under the conditions used so far; and although many attempts have been made in this laboratory to obtain the right combination of factors necessary to excite the sucking reaction of feeding in the absence of a living food organism, no success has been obtained as yet.

Acknowledgments

The authors wish to thank Doctor T. H. Jukes of the Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y., for generous samples of thioctic acid and 8-azaguanine, and Doctor Karl Folkers of

Merck and Company, Rahway, N. J., for the samples of flavotin used in these investigations.

References

1. LILLY, D. M. 1953. Nutrition of carnivorous protozoa. Ann. N. Y. Acad. Sci. **56**: 910.
2. COLLIN, B. 1911. Étude monographique sur les Acinetiens. I. Arch. zool. exp. et gén. **8**: 421.
3. COLLIN, B. 1911. Étude monographique sur les Acinetiens. II. Arch. zool. exp. et gén. **51**: 1.
4. ROOT, F. M. 1914. Reproduction and reactions to food in the suctorian, *Podophrya collini* N.sp. Arch. Protistenn. **35**: 164.
5. LILLY, D. M., P. J. FLEMING, C. F. PEDAGNO & J. P. GILMORE. 1951. Comparative studies on the nutrition, growth and reproduction of suctorian protozoa. Anat. Record. **111**: 62.
6. RUDZINSKA, M. A. 1951. The influence of the amount of food on the reproduction rate and longevity of a suctorian (*Tokophrya infusionum*). Science **113**: 10.
7. RUDZINSKA, M. A. 1951. The effect of overfeeding and starvation on the life span and reproduction of *Tokophrya infusionum*, a protozoan of the class Suctoria. J. Gerontol. **6**: 144.
8. RUDZINSKA, M. A. 1952. The influence of unfavorable conditions on cultures of *Tokophrya infusionum*. Proc. Soc. Protozool. **3**: 10.
9. RUDZINSKA, M. A. 1952. Starvation and longevity of populations in *Tokophrya infusionum*. J. Gerontol. **7**: 493.
10. RUDZINSKA, M. A. 1952. Overfeeding and the life span in *Tokophrya infusionum*. J. Gerontol. **7**: 544.
11. RUDZINSKA, M. A. 1953. Giant individuals and vigor of populations in *Tokophrya infusionum*. Ann. N. Y. Acad. Sci. **56**: 1087.
12. LILLY, D. M. & J. P. GILMORE. 1952. Purines and pyrimidines and abnormal growth of suctorian protozoa. Anat. Record. **113**: 64.
13. LILLY, D. M. & F. J. STERBENZ. 1953. Inhibition and promotion of growth in suctorian protozoa by 8-azaguanine and methyl purines. Proc. Soc. Protozool. **4**: 19.
14. LILLY, D. M., F. J. STERBENZ & V. TARANTOLA. 1953. Growth promotion in carnivorous protozoa by substituted purines. Proc. Soc. Exptl. Biol. Med. **83**: 434.
15. LILLY, D. M. & F. J. STERBENZ. 1953. A cinematographic study of the effect of purines and related compounds on the growth of suctorian protozoa. Anat. Record. **117**: 595.
16. LILLY, D. M., M. J. MOSES & F. J. STERBENZ. 1954. Unpublished observations.
17. RUDZINSKA, M. A. & K. R. PORTER. 1953. An electron microscope study of a protozoan, *Tokophrya infusionum*. Anat. Record. **115**: 363.
18. LILLY, D. M. & F. J. STERBENZ. 1954. Aeration as a factor in the growth of protozoa. J. Protozool. 1 Sup.: 10.
19. KIDDER, G., V. C. DEWEY & R. E. PARKS, JR. 1952. Effect of lowered essential metabolites on 8-azaguanine inhibition. J. Biol. Chem. **197**: 193.
20. STERBENZ, F. J. & D. M. LILLY. 1955. A comparison of the effects of 8-azaguanine and flavotin on *Tetrahymena pyriformis* and *Tokophrya infusionum*. J. Protozool. 2 Sup.: 3.

II. CHEMICAL SUPPLEMENTS PROMOTING GROWTH IN CARNIVOROUS CILIATES*

By Daniel M. Lilly and William H. Cevallos

Department of Biology, St. John's University, Brooklyn, N. Y.

The study of the growth of carnivorous ciliates in two-membered cultures has received much less attention than the growth of ciliates in pure culture. The presence of another species of living organism with all the variables inherent in such a system introduces many difficulties in evaluating results. It is possible, however, by using *Tetrahymena* and other protozoa from pure cultures as food, to grow certain species of carnivorous ciliates in the absence of bacteria with some degree of control over cultural conditions. Thus some interesting growth requirements of these carnivorous forms have come to light. When the problems involved in these two-membered systems have been solved, there is hope that the second step of establishing pure cultures may be successfully approached.

The common hypotrichous ciliate *Stylonychia pustulata* was first established in bacteria-free cultures by supplying *Tetrahymena pyriformis* as food and supplementing the medium with a crude extract of yeast, lettuce, or other plant materials.[1,2] It was found possible to concentrate the necessary supplement by chromatographic methods but, for several years, attempts at substituting combinations of known vitamins or growth factors were unsuccessful.[3] The first progress in this direction came in studies with 8-azaguanine, the well-known guanine analog. Instead of having an inhibitory action such as it was known to have on *Tetrahymena*,[4] the 8-azaguanine was actually growth-promoting for *Stylonychia*. In low concentrations it substituted for the crude yeast extract to the extent of permitting about one half maximum growth.[5] The methyl purines, theophylline, theobromine, and caffeine had a similar effect. In the search for other substances that would increase this growth-promoting effect, a riboflavin analog called flavotin was tested. With this compound other workers[6,7] had previously found potentiation of the carcinostatic action of 8-azaguanine. This potentiation was believed to be due to the inhibition of the enzyme xanthine oxidase. When the combination of 8-azaguanine and flavotin was used in the medium for *Stylonychia* feeding on *Tetrahymena* an improvement was noted, but the growth was still not as good as in the control cultures with yeast extract.

At about this time investigators studying the nutrition of *Paramecium aurelia* had reported a sterol requirement.[8] Sitosterol and stigmasterol,

*This paper, illustrated with lantern slides, was the second of two papers presented at the meeting of the Section, March 12, 1956, on Factors Influencing Growth in Protozoa.

The work described in this paper was supported by a research grant from the National Science Foundation, Washington, D. C.

reported as most effective for *Paramecium*, were therefore added to the medium for *Stylonychia*. Again there was slight improvement in some test cultures but not enough to equal the controls. Meanwhile more progress had been made in this laboratory with a related carnivorous hypotrich, *Euplotes patella*. The first efforts to culture *Euplotes* on a protozoan diet had resulted in the development of an extremely complex supplementary medium containing three crude extracts. One was obtained from yeast, another from liver, and the third from certain species of bacteria.[3,9] The omission of any of these extracts was followed by cessation of growth. Recently, however, it was found possible to replace these crude extracts with known chemical compounds.[10] On the basis of experience with *Stylonychia* the yeast extract was replaced by a mixture of 8-azaguanine and flavotin. The liver extract was found to be replaceable by thiamine. The bacterial factor was more elusive but was eventually replaced by leucovorin (synthetic folinic acid). This combination for *Euplotes* resulted in growth better than that obtained with crude extracts. The same combination has recently been used successfully for *Stylonychia*. The addition of sitosterol improved the medium further so that, for the first time, the growth in these experimental cultures exceeded that in the controls with crude extract.

The replacement of the crude supplement by known chemical compounds still left many questions unanswered concerning their mode of action. The effectiveness of flavotin, however, focused more attention on xanthine oxidase and suggested the use of purine intermediates such as xanthine and hypoxanthine, which can serve as substrates for this enzyme. This slightly different approach has now resulted in a better appreciation of the significance of the supplementary factors in the nutrition of these carnivorous ciliates.

The general methods for culturing carnivorous ciliates have been described previously.[2,3] When the supplementary factors were obtained from crude extracts the food ciliates were grown on proteose peptone dextrose agar slants and then suspended in distilled water for addition to the cultures of *Stylonychia*. Later, when known chemical compounds were used as supplements, it was more important to use a defined medium for the culture of the food organisms. The medium selected was a modification of the well-known Medium A of Kidder and Dewey.[11] The composition of this modification is given in TABLE 1. It differed from the original Medium A in the omission of Tween, an increase in pteroylglutamic acid, and a substitution of one of the inorganic salts. This medium provided excellent growth of *Tetrahymena* but could not be used in full strength for cultures of *Stylonychia*. Apparently food organisms in such large numbers were inhibitory. When diluted to 5 or 10 per cent, however, the medium did not interfere with the growth of the carnivorous ciliates, providing the necessary supplements were added. The *Tetrahymena* medium was used in combination with the supplements in two ways.

An early method was simply to dilute the Medium A to 10 per cent, adding at the same time the necessary chemical supplements. The carnivores and food ciliates were then inoculated simultaneously in a ratio of approximately 1 *Stylonychia* to 100 *Tetrahymena*. Later it was found advantageous to inoculate the food organisms into full strength Medium A and allow them to grow with tubes in a slanted position for four days at 26° C. The experimental combinations of supplements were made up and sterilized separately in 4.5 ml. quantities in 16 × 125 mm. Pyrex culture tubes. The hypotrichs were then inoculated in 0.25 ml. of culture fluid along with 0.25 ml. of the *Tetrahymena* culture. This resulted in a dilution of the Medium A to 5 per cent and a concentration of food ciliates of approximately 4000 per ml. Where reproducibility of the number of food organisms was important it was possible to compare growth of the *Tetrahymena* before inoculation into the *Stylonychia* cultures, using a Bausch and Lomb Spectronic 20 Colorimeter. The 96-hour growth of *Tetrahymena* thus measured in the culture tubes before dilution gave an average optical density of 0.6 at a wave length of 530 mμ.

The growth of experimental cultures was compared with control cultures containing crude extracts. At first an aqueous extract of dried brewer's yeast was employed, as in previous work with *Stylonychia*.[2] For most of the recent work, however, stock and control cultures have been grown in a lettuce medium prepared by boiling 1 gm. of dried powdered lettuce with 500 ml. of distilled water. This crude extract of

TABLE 1

BASAL MEDIUM (MODIFIED MEDIUM A – KIDDER AND DEWEY, 1951)
All Amounts Are Given in μg. per ml. of Final Medium

DL-Alanine	110	Nicotinamide	0.10
L-Arginine	206	Pyridoxine.HCl	1.00
L-Aspartic	122	Pyridoxal.HCl	0.10
Glycine	10	Pyridoxamine.HCl	0.10
L-Glutamic acid	233	Riboflavin	0.10
L-Histidine	87	Pteroylglutamic acid	0.06
DL-Isoleucine	276	Thiamine.HCl	1.00
L-Leucine	344	Biotin (free acid)	0.0005
L-Lysine	272	Choline Cl	1.00
DL-Methionine	248	Thioctic acid*	0.02
L-Phenylalanine	160	Guanylic acid	30
L-Proline	250	Adenylic acid	20
DL-Serine	394	Cytidylic acid	25
DL-Threonine	326	Uracil	10
L-Tryptophan	72	$MgSO_4.7H_2O$	100
DL-Valine	162	$(NH_4)_2SO_4$	0.82
Dextrose	2500	$MnCl_2.4H_2O$	0.50
Sodium acetate	1000	$ZnCl_2$	0.05
Ca pantothenate	0.10	$CaCl_2.2H_2O$	50
K_2HPO_4	1000	$CuCl_2.2H_2O$	5
KH_2PO_4	1000	$FeCl_3.6H_2O$	1.25

*Furnished by the Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y., through the courtesy of T. H. Jukes.

TABLE 2

INFLUENCE OF 8-AZAGUANINE, FLAVOTIN, LEUCOVORIN, AND THIAMINE ON THE GROWTH OF *STYLONYCHIA PUSTULATA*
All Amounts Are Given in μg. per ml.

8-Azaguanine	*Flavotin*	*Leucovorin*	*Thiamine*	*Growth*
0	10	0	0	0
10	0	0	0	0
10	10	0	0	++
1	10	0	0	+
0.1	10	0	0	+
0.1	1	0	0	+
0.01	1	0	0	+
0.001	1	0	0	0
10	10	1	0	+++
10	0	1	0	+
10	10	1	0.5	+++
10	0	1	0.5	++
0	10	1	0.5	++
0	0	1	0.5	+

Each unit represents the average growth in 4 tubes.
Symbols: 0, no growth of hypotrichs; +, significant growth; ++, approximately half maximum growth; +++, approximately three quarters maximum growth; and ++++, maximum growth equal to that in the control tubes.

lettuce, diluted 1:1 with distilled water, proved to be less variable as a source of supplementary factors than the yeast extracts previously used. The growth of *Stylonychia* feeding on *Tetrahymena* in the lettuce medium reached approximately 400 per ml. at the end of 7 days. This was considered maximum growth and was recorded as ++++. In some experiments slightly better growth was obtained as noted in the tables. Three serial transfers were used in experimental cultures to minimize the effect of the previous medium introduced with the inoculum.

After the chemically defined supplementary medium had been developed for *Euplotes*[10] it was soon determined that the same combination was also effective for *Stylonychia*. At about the same time it was also found

that sitosterol had an additional growth-promoting effect. The starting point for this investigation therefore was an attempt to determine the optimum level of each of the chemical compounds in the supplementary mixture.

The effects of different levels of 8-azaguanine, flavotin, leucovorin, and thiamine on the growth of *Stylonychia* are shown in TABLE 2. Although the optimum levels were almost the same as those found for *Euplotes*, the growth was still a little below the controls in lettuce infusion. Flavotin alone was not growth-promoting and, in these experiments, even 8-azaguanine alone had no effect. This was in contrast to earlier experiments where the addition of 8-azaguanine resulted in half-maximum growth. At that time, however, the *Tetrahymena* were not grown in a defined medium, and there was some introduction of diluted yeast extract with the inoculum of *Stylonychia*. Under the conditions of these experiments the combination of the two analogs resulted in half-maximum growth, and this could be improved by the addition of either leucovorin or thiamine.

When the best combination of 8-azaguanine, flavotin, leucovorin, and thiamine was tested with certain sterols, another improvement in growth was obtained. These results are summarized in TABLE 3. Both sitosterol

TABLE 3

INFLUENCE OF STEROLS ON THE GROWTH OF *STYLONYCHIA*
All Amounts Are Given in $\mu g.$ per ml.

Sitosterol	*Stigmasterol*	*Cholesterol*	*Growth*
1	0	0	++++
0.1	0	0	+++
0.01	0	0	+++
0.001	0	0	+++
0	0	0	+++
0	10	0	++++
0	1	0	++++
0	0	10	++
0	0	1	++

All tubes contained: 8-azaguanine 10, flavotin 10, leucovorin 1, thiamine 0.5, guanylic acid 5, K_2HPO_4 250, and KH_2PO_4 250.
Medium A 5 per cent added with the food organisms.

TABLE 4

INFLUENCE OF HYPOXANTHINE ON THE GROWTH OF *STYLONYCHIA*
All Amounts Are Given in μg. per ml.

Theobromine	*Flavotin*	*Hypoxanthine*	*Growth*
50	10	0	++++
50	10	50	++++*
50	0	50	++++
0	10	50	+++
0	0	50	++++*
0	0	0	+

All tubes contained: thiamine 0.5, leucovorin 1, guanylic acid 5, sitosterol 1, K_2HPO_4 50, and KH_2PO_4 50.
Medium A 5 per cent added with the food organisms.
*Better growth than the lettuce infusion controls.

and stigmasterol, which promote growth in *Paramecium aurelia*,[8] were effective in increasing the growth of *Stylonychia* to equal that found in the control cultures in lettuce infusion. Cholesterol, which was not effective for *Paramecium*, had no observable effect on *Stylonychia*. Sitosterol was chosen as the most suitable sterol for further work.

By the addition of the five chemical supplements it was then possible to obtain good growth, but little was known of the mode of action of these supplements. The fact that two antimetabolites best known for their inhibiting action were, under these circumstances, actually growth-promoting, centered attention on the combined effect of 8-azaguanine and flavotin. The theory proposed for the carcinostatic action of this combination emphasized the inhibition by flavotin of xanthine oxidase. The two purine intermediates, xanthine and hypoxanthine, known substrates for this enzyme, were therefore tested in various combinations with the supplements. The growth-promoting effect of hypoxanthine unexpectedly surpassed that of any other compound ever tested. The results are shown in TABLE 4.

In this experiment theobromine was used as a guanine antagonist instead of 8-azaguanine. It was soon found that the antimetabolites were unnecessary if hypoxanthine was present in sufficient concentration. Later, the leucovorin was omitted with no decrease in growth, providing hypoxanthine was available. Thus it became possible to simplify the supplementary medium. Thiamine and sitosterol were still required for maximum growth, and a small amount of guanylic acid was also beneficial. TABLE 5 summarizes some of the experiments that established the optimum level

of these supplements. The best medium found consisted of 5 μg. per ml. of guanylic acid, 50 of hypoxanthine, 0.5 of thiamine, and 1.0 of sitosterol.

A number of other purine compounds were tested for their effect on *Stylonychia* feeding on *Tetrahymena*. Inosine, the riboside of hypoxanthine, was just as effective as hypoxanthine. On the other hand, 2,6-diaminopurine was much less effective, so that growth was reduced to half maximum. Adenylic acid and xanthine were less effective than hypoxanthine. Guanylic acid alone was not beneficial. Indeed, the partial effectiveness of the guanine antagonists suggested at first that an

TABLE 5

THE EFFECT OF SUPPLEMENTS ON THE GROWTH OF *STYLONYCHIA*
All Amounts Are Given in μg. per ml. of Final Medium.

Guanylic acid	*Hypoxanthine*	*Thiamine*	*Sitosterol*	*Growth*
5	0	0.5	1.0	+
5	10	0.5	1.0	+++
5	20	0.5	1.0	++++
5	50	0.5	1.0	++++*
5	100	0.5	1.0	+++
5	50	1.0	1.0	++++
5	50	0.5	1.0	++++*
5	50	0.1	1.0	++++
5	50	0.01	1.0	+++
5	50	0.00	1.0	++
5	50	0.5	1.0	++++*
5	50	0.5	0.5	++++*
5	50	0.5	0.1	+++
5	50	0.5	0.01	++
5	50	0.5	0.00	+

Medium A 5 per cent added with the food organism.
Phosphate buffers 100 μg./ml. used in all tubes.
*Better growth than the lettuce infusion controls.

excess of guanine or guanylic acid might be responsible for the interference. Even with the guanine antagonists, however, concentrations of guanylic acid from 5 to 10 μg. per ml. helped to increase growth. Higher concentrations from 20 to 100 μg. per ml. were definitely inhibitory with or without the antagonist. Uric acid, one of the catabolic products of purine metabolism, in quantities of 10 μg. per ml., reduced growth to half maximum, but otherwise was not particularly toxic.

The interpretation of the results thus far obtained still presents many problems. One difficulty, however, has been considerably clarified. The growth-promoting effects of 8-azaguanine and of flavotin were obviously due to their influence on purine metabolism. At least two known purine intermediates, hypoxanthine and inosine, have now been found to be even more effective. If the action of flavotin was due to the inhibition of xanthine oxidase this could explain an accumulation of hypoxanthine, providing there was available a pathway for the synthesis of this type of purine. That such a pathway might exist was suggested by the effectiveness of leucovorin, the synthetic form of folinic acid, known to be involved in purine synthesis. Furthermore, when exogenous hypoxanthine was available, leucovorin had no observable effect. The apparent synergism between 8-azaguanine and flavotin has not yet been satisfactorily accounted for in *Stylonychia*. If the mechanism postulated by Dietrich and Shapiro[7] for the carcinostatic action was operative here, the accumulation of xanthine and 8-azaxanthine would cause product inhibition of guanase activity. Both guanine and 8-azaguanine then would tend to accumulate. The extent to which the incorporation of 8-azaguanine into the nucleic acids of *Tetrahymena* affected the *Stylonychia* feeding on them has not been determined. Presumably, incorporated 8-azaguanine would eventually become available to the carnivorous ciliates.

The principal difficulty arises from attempts to evaluate the effectiveness of individual purines when we have practically no knowledge of the types and concentrations of compounds actually furnished by the digestion of the food organisms. The established facts are that *Tetrahymena* grown in the usual media is inadequate as food, but becomes adequate when the medium is supplemented with additional sterol, thiamine, guanylic acid, and hypoxanthine. The supplement of hypoxanthine is not required if a guanine antagonist, flavotin, and leucovorin are substituted. It is still not clear whether the effects on metabolism occur exclusively in *Stylonychia* or partly in the food organism. *Tetrahymena* apparently is not affected by flavotin[12] and does not require folinic acid, providing folic acid is available.[13] *Tetrahymena* does have its own requirement for exogenous guanine or guanylic acid,[11] but apparently *Stylonychia* requires slightly more guanylic acid with or without the guanine antagonist. One conclusion that can be drawn is that purine

metabolism is quite different in the two species and the supplements in some way compensate for the difference.

This information may prove valuable for further work on the nutrition of *Stylonychia* and other species of ciliates. Some of the peculiarities of one phase of metabolism are now known. This may aid in the solution of the next step, the establishment of axenic cultures. Large numbers of bacteria-free organisms can now be obtained for more extensive experiments based on experience with this and other species. The fact that *Stylonychia* has several cultural characteristics in common with other species of protozoa offers new hope for a solution to the major difficulties that have so far prevented the controlled culture of many common ciliates.

Acknowledgments

The authors wish to thank T. H. Jukes of the Lederle Laboratories, American Cyanamid Company, Pearl River, N. Y., for the generous samples of thioctic acid and 8-azaguanine, and Karl Folkers of Merck and Co., Inc., Rahway, N. J., for the samples of flavotin used in these investigations.

References

1. LILLY, D. M. 1940. A supplementary growth factor in the nutrition of carnivorous ciliates. Anat. Record. **78**: 40.
2. LILLY, D. M. 1942. Nutritional and supplementary factors in the growth of carnivorous ciliates. Physiol. Zoöl. **15**: 146.
3. LILLY, D. M. 1953. Nutrition of carnivorous protozoa. Ann. N.Y. Acad. Sci. **56**: 910.
4. KIDDER, G. W. & V. C. DEWEY. 1949. The biological activity of substituted purines. J. Biol. Chem. **179**: 181.
5. LILLY, D. M., F. J. STERBENZ & V. TARANTOLA. 1953. Growth promotion in carnivorous protozoa by substituted purines. Proc. Soc. Exptl. Biol. Med. **83**: 434.
6. SHAPIRO, D. M. & R. A. FUGMANN. 1952. Combination chemotherapy of cancer with 8-azaguanine and a riboflavin analogue. Proc. Soc. Exptl. Biol. Med. **81**: 239.
7. DIETRICH, L. S. & D. M. SHAPIRO. 1953. Combination chemotherapy of cancer: potentiation of the carcinostatic action of 8-azaguanine by a riboflavin analog. Cancer Research **13**: 699.
8. VAN WAGTENDONK, W. J., R. L. CONNER, C. A. MILLER & M. R. R. RAO. 1953. Growth requirements of *Paramecium aurelia* var. 4, stock 51.7 sensitives and killers in axenic medium. Ann. N.Y. Acad. Sci. **56**: 929.
9. LILLY, D. M. & S. M. HENRY. 1954. Modification of the medium for the bacteria free culture of *Euplotes*. Anat. Record. **120**: 814.
10. LILLY, D. M. & S. M. HENRY. 1955. Chemical factors required for the bacteria free culture of *Euplotes*. Anat. Record. **122**: 427.
11. KIDDER, G. W. & V. C. DEWEY. 1951. The biochemistry of ciliates in pure culture. Biochemistry and Physiology of Protozoa. ed. Lwoff, Academic Press, New York. N. Y.
12. STERBENZ, F. J. & D. M. LILLY. 1955. A comparison of the effects of 8-azaguanine and flavotin on *Tetrahymena pyriformis* and *Tokophrya infusionum*. J. Protozool. 2 Sup. : 3.
13. KIDDER, G. W., V. C. DEWEY & R. E. PARKS, JR. 1951. Growth promotion in *Tetrahymena* by folic acid analogs. Proc. Soc. Exptl. Biol. Med. **78**: 88.

SECTION OF MATHEMATICS AND ENGINEERING

HIGH-SPEED PHOTOGRAPHY AND THE NATURAL SCIENCES*

By John H. Waddell

Fairchild Camera and Instrument Corp., Jamaica, N. Y.

The purpose of high-speed motion picture photography is to magnify time in order to reproduce motion at a rate at which it can be detected or made more perceptible. This purpose is accomplished by taking pictures at a high rate of speed and projecting them at a lower rate. The process is used extensively by the engineer as well as by the biochemist, the botanist, the biologist, the physician, and the surgeon. New vistas for its use are constantly opening up, and it is predicted that with the advent of new, low-cost, rotating-prism cameras that can be hand-held, the use of the high-speed motion picture camera will become an integral part of instruction in many branches of higher education, an increasingly important instrument for research, and the outstanding tool for obtaining positive information about motion.

Photographic studies of the movement of fauna were made as early as the 1870's, and of flora in 1915. Until 1937, the two basic methods used for these studies were still pictures and time-lapse motion pictures, and the use of high-speed motion pictures followed these. Some of the pictures are used for aesthetic purposes and amusement, but the majority of them serve a more practical purpose. The stills are used primarily for records, while the motion pictures are used as a means for extensive scientific studies.

Pathé, of France, released colored motion pictures showing the opening of flowers in 1915. These pictures were made by the time-lapse method, and each frame was hand-tinted and individually matched so that the flowers would look natural on projection. Not one, but many films were made by this method—a colossal undertaking.

Time-lapse pictures of plants show that they behave much as do the members of the animal kingdom, with the exception that their movements are much slower. They provide necessary information for the study of such things as rate of growth, reaction to the stimuli of sound and light, the effects of chemical feeding and fertilization, the results of disease, and the action of carnivorous plants such as the Venus fly-trap. Much of this type of work was originally done at Rutgers University, New Brunswick, N. J. Today, John Nash Ott, of Winnetka, Ill., is probably doing the most outstanding work in this field. He is producing short subjects for television, making sequences for the Walt Disney *True Life Adventure* series, and performing scientific studies for horticulture.

Eadweard Muybridge, in the early days of the slow, wet photographic

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on March 16, 1956.

plate, made a comprehensive series of pictures showing the motions of humans and animals. He used as his subjects man, dogs, horses, elephants, snakes, and several other animals. He had them perform nearly all the bodily motions of which they were capable. He placed a number of these pictures, showing progressive movements, in consecutive order on the outside of a rotating drum that had slits in it through which the pictures were viewed, in this way producing the earliest form of a motion picture. Later he found that by using grids it was possible to measure the deflection of limbs and muscles. His illustrations, along with the conclusions drawn from his photographic studies, were published in a series of volumes on animal locomotion. When one considers the slow photographic plates, the slow lenses, and the limited lighting facilities that existed in Muybridge's time, his work is of a classic nature. The facts that his book *The Human Figure in Motion*[1] has been recently published and that a companion work, *Animal Locomotion*[2] is now in press are indication that the unique quality of his work is still appreciated.

Popular interest in the behavior of animals is demonstrated by the enthusiastic reception of Disney's *True Life Adventure* series. It is only recently, however, that Disney has used pictures of more rapidly moving subjects, taken at 64 to 128 pictures per second. Even this rate, however, is not fast enough to show many of the things in which the scientist is interested.

The high-speed motion picture camera has been used to explore many fields of the natural sciences. Henry Lester, in 1955, illustrated muscular action in his pictures of the snake striking, as has J. H. Waddell in his ballet and tennis studies taken in 1947. The flight of birds and bees, the landing and the take-off of a fly, and the jumping of a frog under various stimulae have been photographed by a number of technicians and engineers. Interesting subjects that might be investigated are: (1) comparisons between the leaping of insects such as the grasshopper, the cricket, and the flea; (2)the method of locomotion of a snake; and (3)the synchronization of the legs of a centipede or a millepede.

Considerable research has been done in the sphere of medicine by the use of high-speed motion photography. This includes studies of the action of the vocal cords, the action of the eardrum, and the performance of the heart.

The study of speech is a very important function of the communications industry, particularly telephony. It is a known fact that each individual has a characteristic speech formation that gives him a distinguishable voice, recognizable over a telephone. It was therefore quite natural for the Bell Telephone Laboratories, New York, N. Y.,* to start an extensive series of high-speed motion pictures to determine the reason for speech differences. It was found that facial contours and such cavi-

*There are available two 400-foot films on the subject, obtainable from the Bureau of Publication of the Bell Telephone Laboratories, New York, N. Y.

ties as the nasal tract, mouth, esophagus, and larynx, in conjunction with the vocal cords, are all contributing factors. For many years previously, it had been assumed that the vocal cords behave much as rubber bands do, that the cords were stretched as the frequency of vibration increased, the notes going higher up the scale. Also, it was assumed that the vocal cords of a woman would be taut and elongated when compared to those of a male, but both of these suppositions were proved false.

These early studies were made at the rate of 4,000 pictures per second. An incadescent filament was cooled with a water cell and its image was projected on the vocal cords by the use of a lens and a throat mirror. When the subject was requested to say "Ah," the various parts of the throat cavity aided the photographer by getting out of the way so that the vocal cords could be observed. The female subject was of very little help in these studies as she had a greater tendency to gag on the throat mirror, and New York law does not permit anesthetizing the throat by nonmedical people. Another interesting and rather amusing thing showed up during these voice studies when helium was used instead of air. The cords photographed the same as they did when air was used, but there was a decided eunuchization of the male voice. When the gas pressure was increased by the use of a plastic gag, the voice became virile again. This proved that the density of the gas controls the voice tone.

The study of speech was continued at the University of Minnesota, Minneapolis, Minn., by taking pictures at rates up to 8,000 per second. These studies are responsible for the necessity of rewriting the textbooks on vocal cords, since previous teaching was contrary to these disclosures.

The hearing process is another function of the communications industry, so it is not surprising that the first high-speed motion picture studies of the eardrum were also made by Bell Telephone Laboratories. Cats and guinea pigs were the first subjects, and cadavers were used later. The side of the head was sectioned and the eardrum viewed under various stimulae, such as explosions and low- and high-level sounds of varying frequencies. More extensive studies along these lines were carried on at Harvard University, Cambridge, Mass., by Von Bekesy in 1946.

In 1944 the first high-speed motion pictures were taken of the muscular action of a dog's heart. This pioneering work was done by Myron Prinzmetal at the Cedars of Lebanon Hospital, Los Angeles, Calif. It is described fully in the book entitled, *The Auricular Arrhythmias*.[4] The first high-speed motion pictures of the human heart were taken at St. Clair's Hospital, New York, N.Y., by J. H. Waddell, whose techniques were later adopted by Prinzmetal and his associates.

The fine high-frequency amplitude waves on the cardiogram always intrigued the medical profession. It was recognized that any irregularities of these waves are closely associated with the malfunctioning of the heart, or heart disease. That the heart beats approximately 78 times

a minute is discernible even to the layman, but it is not evident to him that the heart is in a constant succession of motion. It was not until the first high-speed motion tests were made that it was learned that a high-frequency pulsing of the auricles and ventricles takes place in the pericardial sac. Prinzmetal's early studies showed this constant muscular action as well as the ability of various drugs to produce either rhythmic or arrhythmic action.

Prinzmetal also took divided-image pictures to show the cardiogram simultaneously with the mechanical heart action, which gave valuable additional knowledge. More recently he has used a dual-channel cathode-ray oscilloscope to get high-speed motion pictures of the heart. Burch, at Tulane University of Louisiana, New Orleans, La., has been doing considerable cinematography of the heart, and Tenney and Honig, at Strong Memorial Hospital, Rochester, N.Y., have been using the high-speed motion picture camera to make vector analyses of the action of aconite on the heart. X-ray motion pictures have been made at Mount Sinai Hospital, New York, N.Y., by Underglider of Equitable Life Insurance Company, New York, N.Y., and by Rehman of the University of Southern California, Los Angeles, Calif. Other organs and parts of the body, as well as the heart, are under study by Ramsey and Sidney Weinberg of Strong Memorial Hospital, Rochester, N.Y. Also, extensive work has been done in Germany on complete body processes.

In any attempt to take high-speed motion pictures of the heart action it is imperative that precautions be taken to eliminate the hazard of burning the tissues. To get sufficient lighting, high-intensity lamps are focused on the subject, and it is only logical that these light sources should first be cooled. This is done by means of water cells. It is not possible to get pictures of a normal heart under the conditions of operative procedures, as the subject is under the influence of anesthetics and drugs. In an attempt to approximate the normal heart, one of the objectives of the X-ray cinematography has been to increase the speed of the system so as not to cause an overdose of X rays to the subject. The more recent work along these lines is being done with image amplifiers, such as those developed by Philips N.V.D. of Eindhoven, Netherlands, and the Westinghouse Electric and Manufacturing Corporation, of Baltimore, Md. It seems feasible that the ideal way to take high-speed motion pictures for these studies might be to use C. Slack's microsecond pulse X ray, synchronized with the camera.

Some studies that are impending include: (1) the flow of blood when viewed through a microscope; (2) the movement of spermatozoa; and (3) the crystallization process that may occur on the body.

References

1. MUYBRIDGE, EADWEARD. 1901. The Human Figure in Motion. Chapman & Hall, London, Eng. (Reprinted. Dover Publications. New York, N.Y. 1955).

2. MUYBRIDGE, EADWEARD. 1899. Animals in Motion. Chapman & Hall. London, England. (In Press, Dover Publications. New York, N. Y. 1956.)
3. FARNSWORTH, D. W. 1940. High Speed Motion Pictures of the Human Vocal Cords. Bell Lab. Record 18: 203-208.
4. PRINZMETAL, MYRON. 1952. The Auricular Arrhythmias. Charles C. Thomas. Springfield, Ill.

SECTION OF PSYCHOLOGY

SYMPTOM MECHANISMS IN PSYCHIATRIC PATIENTS*

By Robert B. Malmo

Laboratory for Psychological Studies, Allan Memorial Institute of Psychiatry, McGill University, Montreal, P.Q., Canada

Unfortunately, space does not permit me to present a detailed account of a theoretical consideration of pathological anxiety in psychiatric patients. I shall simply outline the main argument and direct the reader elsewhere for a more complete exposition.

I have previously attempted to construct a neuropsychological model of "pathological" anxiety. By "pathological" anxiety I mean a chronic clinical condition characterized by overarousal to stimulating situations. The term refers to the probability that overarousal will occur upon stimulation, and does not refer to actual feelings or reactions.

The model is built upon the basis of Eccles' recent finding that central inhibition has electrical properties opposite to those of excitation,[1] and upon the recent discoveries concerning reticular systems in brain stem and thalamus, or what have been called the "nonspecific projection systems" (NPS).

It is the main thesis of the present paper that anxiety may be accounted for in terms of deficiency in the inhibitory part of the NPS.

Experimental Studies with Psychiatric Patients

My theory is largely based on the findings of our staff from our objective studies of psychiatric patients. Our data suggest that one dimension in which a meaningful difference between patients and controls has been established experimentally is the dimension of response intensity or arousal. This appears true of psychoneurotic patients in general and of patients with clear symptoms of anxiety in particular.

In fact, of the patients whom we have studied, the group in which anxiety stood out as a predominate symptom appeared to be the most highly responsive of all. We defined anxiety as a pathological state of such severity that work efficiency was interfered with and medical advice was sought, and which was characterized by one or more of the following complaints: persistent feelings of tension or strain, irritability, unremitting worry, restlessness, inability to concentrate, feelings of panic in everyday-life situations.

In a study with pain as standard stimulus the following physiological measures showed significantly greater reaction in anxiety patients than in other psychiatric patients: finger movement (and number of voluntary pressures to indicate pain), neck-muscle activity, deviation in amplitude

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on March 19, 1956.

and rate of respiration throughout the test, respiratory irregularities occurring at time of stimulation, and heart-rate variability. In a different study with a perceptual test and a Luria-type recording from the left hand, finger movement was significantly more irregular in anxiety patients than in other psychiatric patients.

To repeat an earlier statement, these findings indicate that under standard conditions of stimulation psychoneurotics are more reactive than controls, and that patients with anxiety predominating in the symptom picture are the most responsive of all.

Need for "Standard Stress" in Demonstrating Differences Between Patients and Controls

Another question we sought to answer was whether a certain level of arousal must be reached in order to demonstrate differences between patients and nonpatients or whether such differences could be best obtained under resting, "basal" conditions. From a review of the literature prior to conducting our own experiments we were led to suspect that some stimulation would be necessary because experiments carried out under resting or "basal" conditions had usually yielded negative or inconclusive results.

Our findings did indeed clearly show that, in differentiating between patients and controls, some form of stimulation was definitely superior to merely taking records under resting conditions. This has been demonstrated for blood pressure (Malmo and Shagass,[2] p. 89) for muscle potentials in motor tasks (Malmo, Shagass, and Davis,[3] pp. 54 and 59 ff.) and again for muscle potentials in two separate investigations of startle (Malmo, Shagass, and Davis,[4] p. 327; and Davis, Malmo, and Shagass,[5] p. 181). The only measure we have found for good discrimination between patients and controls under "resting" conditions is frontalis-muscle potentials.[6] We know, however, that "resting" conditions associated with a testing session are by no means basal, and that, for example, significantly lower blood-pressure readings may be obtained from patients resting quietly on the ward (Malmo, Shagass, and Heslam,[7] p. 170) than in the so-called "resting conditions" of our experiments.

"Specific" versus "nonspecific" stimulating situations. In producing higher levels of arousal in patients is it necessary to present material to which patients are specifically sensitized, or is it possible to demonstrate the difference between patients and controls by employing the same standard stimulating situation for all subjects? Our experiments clearly show that the latter is true. It is not necessary to present the patients with words or situations that have special meanings for them in order to produce more arousal in them than in controls.

As an example of a "specific-complex" technique of producing high-level arousal, Luria[8] employed the method of controlled association in

which he compared motor reaction to "critical" words (those words especially arousing to the subject because of their association with specific life experiences) with reaction to indifferent words. Our situations, on the other hand, were chosen for their general arousal value, and we sought to avoid situations that would have special meaning for particular individuals.

With this point especially in mind we devised our standard situation of painful stimulation, because of the nearly universal avoidance reaction to pain. In order to permit more generalized conclusions, we also employed standard situations other than pain. One study is of especial interest because we reproduced the essential features of Luria's procedure, only substituting a series of size discriminations for the series of verbal stimulations Luria employed. Conclusions from these experiments were as follows:

(1) All measures of motor activity recorded during performance of speeded size discrimination yielded reliable differences between patients and controls. In every instance there was evidence of greater physiological disturbance in the patients. The measures employed may be distinguished as skeletal-motor (motor control, muscular tension) and autonomic (systolic blood pressure).

(2) These differences in motor activity were manifested even though psychoneurotics, acute psychotics, and controls were practically identical with respect to perceptual performance (Malmo, Shagass, Belanger, and Smith,[9] p. 546 ff.).

These results led us to question certain views concerning determinants of higher arousal levels in psychoneurotics. I refer to the assumption that in a particular psychoneurotic, as a product of his own individual life experiences, certain situations (or words, say, in the association experiment) have acquired such high arousal-producing values that they produce a marked physiological reaction. Strong emphasis is laid on the arousal-value of the situation, and the amount of physiological disturbance is viewed as commensurate with the special significance the situation has for the patient, by virtue of prior learning. This view, in its extreme form, does not consider the possibility of a defective arousal mechanism. It assumes that the observed physiological reaction is appropriate to the arousal-value of the situation, that it is the reaction of "normal" motor mechanisms to stress of extremely high intensity.

This view may well be questioned, however, because it does not appear to fit with clinical observations. Cameron,[10] p. 57, has written as follows: "It will be noted that nearly all such patients [with anxiety states] complain that they cannot go into crowded places or into any situation where sustained efforts will be required of them. Their symptoms are made more severe by anything which elicits emotional reactions, such as altercations or participating in a discussion of illness. Nearly all find, at least at first, that their symptoms are increased by visiting

their former places of employment or meeting fellow-workers. In other words, their symptoms are exacerbated by anything which serves to increase tension. *Emphasis should be placed upon the fact that their symptoms are elicited or intensified, not primarily by the reactivation of any conflict situation which may exist, but literally by everything in the course of the day which serves to increase tension.*" (Italics mine.)

In therapy, relaxants of various kinds are advised to "damp" the "autonomous" reaction before proceeding with psychotherapy (Cameron,[11] p. 142 ff.; and Tyhurst and Richman[12]).

Strong auditory stimulation. Strong auditory stimulation served as a third and very different kind of standard stimulation for comparing patients and controls. Two separate studies with quite different auditory stimuli, one study with induced tension and the other without it, agreed in showing that the most reliable difference between anxious patients and controls was in "after response" following the period of primary reflex-startle reaction.[4,5]

As a matter of fact, it was the first experiment on auditory stimulation that led us to consider the reticular systems in relation to our problem. When we called H. H. Jasper's attention to the fact that the main difference between patients and controls was in the "after-response" period he showed us an unpublished paper[13] and suggested that this was the kind of difference he would expect if the reticular systems were involved. In reviewing the literature we were impressed by the reports of abolishing after-discharge from stimulation in the reticular systems.[4]

Experiments on the reticular systems repeatedly showed that the NPS characteristically inhibits by reducing or abolishing after-discharge. Turning back to our data on patients, it appeared that inhibitory mechanisms normally effective in reducing after-response were inoperative or defective in patients with pathological anxiety. These data, therefore, seemed to provide empirical support for an interpretation of anxiety as defective inhibition by mechanisms in the reticular systems.

Although more significant differences between patients and controls were obtained for the "after-response" period, patients in the experiment with the less intense stimulus showed a significantly higher immediate reaction as well. It is important to note that a simple unstructured stimulus, producing reflex reaction, differentiates anxious patients from matched normal controls.

The Nonspecific Projection System as Part of a Neuropsychological Model to Conceptualize the Problem of Anxiety

Since 1950, work on the reticular systems has progressed so rapidly that Hebb,[14] p. 250, has proposed them as mediators of arousal function. This is in line with our earlier suggestion that the symptom of anxiety in psychoneurosis may be due to defective regulation by the reticular systems. Using experimental, clinical, and neurophysiological data I

have attempted to construct a neuropsychological model that will be useful as a frame of reference in seeking to determine what kind of defect in the regulation of arousal level may be responsible for symptoms of pathological anxiety in psychiatric patients.[15]

References

1. MALMO, R. B. 1954. Eccles' neuropsychological model of the conditioned reflex. Can. J. Psychol. **8**: 125.
2. MALMO, R. B. & C. SHAGASS. 1952. Studies of blood pressure in psychiatric patients under stress. Psychosom. Med. **14**: 82.
3. MALMO, R. B., C. SHAGASS & J. F. DAVIS. 1951. Electromyographic studies of muscular tension in psychiatric patients under stress. J. Clin. Exper. Psychopath. **12**: 45.
4. MALMO, R. B., C. SHAGASS & J. F. DAVIS. 1950. A method for the investigation of somatic response mechanisms in psychoneurosis. Science. **112**: 325.
5. DAVIS, J. F., R. B. MALMO & C. SHAGASS. 1954. Electromyographic reaction to strong auditory stimulation in psychiatric patients. Can. J. Psychol. **8**: 177.
6. MALMO, R. B. & A. A. SMITH. 1955. Forehead tension and motor irregularities in psychoneurotic patients under stress. J. Personality. **23**: 391.
7. MALMO, R. B., C. SHAGASS & R. M. HESLAM. 1951. Blood pressure response to repeated brief stress in psychoneurosis: a study of adaptation. Can. J. Psychol. **5**: 167.
8. LURIA, A. R. 1932. The Nature of Human Conflicts. Liveright. New York, N.Y.
9. MALMO, R. B., C. SHAGASS, D. J. BELANGER & A. A. SMITH. 1951. Motor control in psychiatric patients under experimental stress. J. Abnormal Social Psychol. **46**: 539.
10. CAMERON, D. E. 1944. Autonomy in anxiety. Psychiat. Quart. **18**: 53.
11. CAMERON, D. E. 1950. General Psychotherapy. Grune & Stratton. New York, N.Y.
12. TYHURST, J. S. & A. RICHMAN. 1955. Clinical experience with psychiatric patients on reserpine—preliminary impressions. Can. Med. Assoc. J. **72**: 458.
13. JASPER, H. H. 1949. Diffuse projection systems: the integrative action of the thalamic reticular system. EEG Clin. Neurophysiol. **1**: 405.
14. HEBB, D. O. 1955. Drives and the C.N.S. (Conceptual nervous system). Psychol. Rev. **62**: 243.
15. MALMO, R. B. A theory of anxiety. To be published.

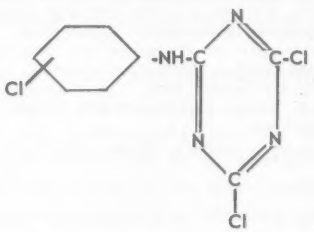
DIVISION OF MYCOLOGY

STUDIES ON THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF THE *s*-TRIAZINES*

By H. P. Burchfield

Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

The discovery by Wolf, Schuldt, and Baldwin[6] that many *s*-triazine derivatives are capable of inhibiting the germination of fungus spores provided an excellent opportunity to study the effects of halogen substitution and isomerism on biological activity, since these compounds are stable in aqueous media and undergo relatively simple reactions with metabolic intermediates containing amino and sulfhydryl groups. Furthermore, a sensitive analytical method applicable to many of these compounds was developed that made it possible to study their uptake from dilute aqueous solution by fungus spores, and their reaction rates with amino acids and peptides.[2] Although more than 80 compounds were prepared and evaluated as fungicides, these present studies were confined to 2,4-dichloro-6-anilino-*s*-triazine and derivatives with a chlorine atom in the *ortho*, *meta*, and *para* positions of the benzene ring, since this group was found to be highly effective in inhibiting spore germination and in controlling certain foliage diseases of higher plants.



It was assumed that the fungicidal activity of these compounds was caused by the ability of the active halogen atoms in the triazine ring to undergo substitution reactions with nucleophilic metabolites, for when one of the halogens was replaced by an anilino or phenoxy group, fungitoxicity decreased considerably while, when both were replaced, it vanished altogether.[5] This is in accordance with the *in vitro* experiments of Fierz-David and Matter[3] who showed that replacement of the first halogen takes place readily at room temperature, while replacement of

*This paper, presented at a meeting of the Section on March 23, 1956, was abstracted from a thesis entitled *Uptake of Derivatives of 2,4-Dichloro-6-anilino-s-triazine and Derivatives by Conidia of* Neurospora sitophila *and Their Reactions with Metabolic Intermediates*, presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University, New York, N. Y. This paper was read instead of the paper "Some Mechanisms Concerned in the Action of Fungicides," which was to have been given by Robert G. Owens.

the second requires the application of heat. Thus it is necessary to consider the effects of 2 types of halogen substitution: (1) the labile chlorine atoms on the triazine ring that probably represent the functional groups, and (2) those atoms on the benzene ring that exert a modifying effect either through influencing uptake by fungus spores or by altering chemical reactivity.

In order to assess the relative importance of these modifying effects, the uptake of the 4 compounds from dilute aqueous solution by conidia of *Neurospora sitophila* (Mont.) Shear and Dodge was first investigated. When the compounds were present in amounts corresponding to 500 μg. per gram of spores, removal was very rapid for the first few minutes, but thereafter leveled off to reach a maximum, after which the amount of triazine in the aqueous solution apparently increased. The unsubstituted compound and the derivative with a chlorine atom in the *ortho* position of the benzene ring were taken up less rapidly than the *m*-chloro and *p*-chloro derivatives but, in all cases, over 80 per cent of the available material was removed from the aqueous solution at maximum uptake. When the number of spores was reduced so that the amount of triazine was equivalent to 1,000 μg. per gram of spores, the period of release was followed by another period of uptake, suggesting that uptake occurs in 2 stages. The first stage was observed when the amount of triazine with respect to the number of spores was low, and the second was only observed when a considerable amount of triazine remained in the aqueous phase at maximum uptake. Evidently these compounds are able to alter the permeability of spore membranes so that part of the material that is accumulated can diffuse back into the aqueous phase. These results were confirmed by the observation that, on treatment with the *o*-chloro derivative, spores grown on a medium containing $P^{32}O_4^{---}$ released compounds containing radioactive phosphorus. Since spores treated at this dosage rate were still able to germinate, it was evident that the fungitoxicity of these compounds could not be ascribed to their ability to alter the permeability of the spore membranes.

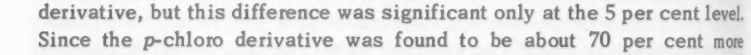
The effect of halogen substitution and isomerism on the amounts of these materials that could be taken up by conidia of *N. sitophila* under equivalent conditions was established by treating spores with 6 successive doses of each of the 4 chemicals and measuring cumulative uptake. It was found that substitution of a chlorine atom in the *ortho* position of the benzene ring increased uptake by about 30 per cent, while substitution in the *meta* or *para* positions increased uptake by 80 per cent. When small numbers of spores were exposed to comparatively large doses of the 4 compounds in single treatments, it was found that the difference between the *meta* and *para* isomers was accentuated, so that the order of uptake by spores became *p*-chloro > *m*-chloro > *o*-chloro > unsubstituted. The apparent fungitoxicity of the 4 compounds to conidia of *N. sitophila*

was found to decrease in the same order in tests carried out by the glass slide-germination procedure.[1] It became evident, therefore, that differences in observed toxicity were primarily caused by differences in the amounts of the compounds that could be taken up under equivalent conditions. It may be that this can be accounted for in part by the Overton-Meyer partition theory,[4] since the compounds that were taken up most readily were found to be least soluble in water.

If this is true the toxicities of the 4 compounds should be approximately equivalent when ED50 values are determined on the basis of the amount of material taken up by the spores rather than on the applied dosages. In order to confirm this, spores were treated with a series of successive doses of the *p*-chloro and *o*-chloro derivatives, and the cumulative uptake was determined for each treatment. The spores were then transferred to a triazine-free medium, and percentage germination was determined. The average ED50 value obtained for the 2 compounds in 5 independent experiments was 1,600 μg. of compound per gram of spores. The *o*-chloro derivative was found to be about 8 per cent more toxic than the *p*-chloro derivative, but this difference was significant only at the 5 per cent level. Since the *p*-chloro derivative was found to be about 70 per cent more toxic than the *o*-chloro derivative in the glass slide-germination test,[1] where results are calculated on the basis of the dose applied, it appears safe to conclude that differences in apparent toxicity within this series are caused primarily by differences in the amounts taken up rather than by changes in intrinsic toxicity.

Since halogen substitution on the benzene ring would be expected to alter the chemical reactivities of these compounds as well as the amounts that could be taken up by spores, comparative reaction-rate studies were made *in vitro*, using cysteine and glycine as typical substrates.

It was assumed that the reaction would involve replacement of one of the halogen atoms on the triazine ring by compounds containing sulfhydryl or amino groups according to the scheme



Cysteine was found to react 100 times more rapidly than glycine, so it is apparent that combinations with molecules containing sulfhydryl groups would be favored on a competitive reaction-rate basis. This cannot be the only mechanism by which spore germination is prevented,

however, since it was found that inhibition can be only partly reversed by the addition of cysteine or glutathione to the medium. Subsequent experiments have shown that other small molecules such as proline and *p*-aminobenzoic acid can also combine very rapidly with the triazines.

When second-order rate constants for the 4 triazine derivatives were compared it was found that their chemical reactivities decreased in the order *o*-chloro > *m*-chloro > *p*-chloro > unsubstituted, with both cysteine and glycine as substrates. Thus halogen substitution in the benzene ring increases the chemical reactivity of the halogens in the triazine ring. This is probably an inductive effect, since activation diminishes progressively as the halogen is moved away from the triazine ring.

Chemical reactivity is doubled when a chlorine atom is substituted in the *o*-position of the benzene ring but, at least in the case of *N. sitophila*, this is not the major cause of differences in apparent fungitoxicity. Thus the *o*-chloro derivative is 36 per cent more reactive with cysteine than the *p*-chloro derivative, but the ED50 value is only 8 per cent lower. Larger differences in performance might be expected between compounds where groups such as *p*-nitro, *p*-methoxyl, *etc.* are substituted in the benzene ring, since the activating effects would be different in sign, and much larger differences in chemical reactivity would be expected. It seems likely that an optimum degree of reactivity must exist for toxicants that act as general alkylating agents, since a highly reactive compound might hydrolyze in the environment before it could become effective, while a relatively unreactive material might not be able to combine with essential metabolites at rates greater than they could be replaced. In a forthcoming paper by Schuldt and Wolf [5] a number of compounds are described that might provide examples of these types, but it is not possible to assess the importance of these effects without more information on their relative reactivities and the amounts that can be taken up by spores. In the group of compounds under current investigation, differences in reactivity are apparently of secondary importance. It is likely, however, that reactivity of this general order is prerequisite for compounds that act primarily as alkylating agents, and major departures from this order in either direction would lead to considerable differences in innate toxicity.

In experiments made with conidia of *N. sitophila*, the amount of these materials that can be taken up by spores is a more critical factor than reactivity in determining apparent toxicity by the glass slide-germination test. In this technique the spores are immersed in solutions that in some cases contain from 7.5 to 52.5 times the amount of triazine required to inhibit germination, so the amount that is taken up, rather than the supply, is limiting. These observations might not necessarily apply to all species of fungi, however, since the order of apparent toxicity of these compounds to *Alternaria solani* (Ell. and Mart.) Jones and Grout

was found to decrease in the order *o*-chloro > *p*-chloro > *m*-chloro > unsubstituted. Thus the *para* isomer is more effective than the *ortho* isomer against conidia of *N. sitophila*, while the reverse is true in the case of *A. solani*. Whether this inversion is due to differences in uptake or other physicochemical factors has yet to be investigated. Nevertheless, these observations show that it is dangerous to generalize about the activity of any toxicant without also taking into account the characteristics of the organism to which it is applied.

References

1. AMERICAN PHYTOPATHOLOGICAL SOCIETY COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS. 1943. The slide-germination method of evaluating protectant fungicides. Phytopathology. **33**: 627-632.
2. BURCHFIELD, H. P. & E. E. STORRS. A colorimetric method for the determination of 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine and related compounds. In preparation.
3. FIERZ-DAVID, H. E. & M. MATTER. 1937. Azo and anthraquinonoid dyes containing the cyanuric ring. J. Soc. Dyers Colourists. **53**: 424-436.
4. RICH, S. & J. G. HORSFALL. 1952. Lipid solubility and fungitoxicity. Phytopathology. **42**: 17.
5. SCHULDT, P. H. & C. N. WOLF. Fungitoxicity of substituted triazines. In preparation.
6. WOLF, C. N., P. H. SCHULDT & M. M. BALDWIN. 1955. *s*-Triazine derivatives — a new class of fungicides. Science. **121**: 61-62.

SECTION OF OCEANOGRAPHY AND METEOROLOGY

TEN MONTHS OF OPERATIONAL NUMERICAL WEATHER PREDICTION*

(Abstract of the Paper)

By Phillip D. Thompson

Development Section, Joint Numerical Weather Prediction Unit, Suitland, Md.

The Joint Numerical Weather Prediction Unit, a combined activity of the United States Weather Bureau, Air Force, and Navy, was established in July 1954. It issued the first prognostic weather maps on a routine operational basis on May 6, 1955.

The predictions are formulated in the unit headquarters in Suitland, Md., by means of an IBM 701 electronic computer that integrates numerically the prediction (vorticity) equations that have been derived from the fundamental meteorological equations by J. Charney and others.

Forecasts are currently being prepared on the basis of two theoretical models, but other models are being developed and tested. The three-level (900, 700, 400 mb.) baroclinic model devised by Charney and Phillips and based on Rossby's principle of conservation of potential vorticity is used to produce 12-, 24-, and 36-hour prognostic maps once a day from 1000 EST data. An iteration time of 30 minutes is used, and the forecasts are completed by 1730, 1800, and 1830 EST respectively.

A barotropic forecast for 500 mb. (based on the principle of conservation of absolute vorticity) is also prepared daily for 24-, 48-, and 72-hours for almost the entire Northern Hemisphere.

The interpolation of data for the grid used is carried out by the computer, which performs an objective analysis by quadratic interpolation, smooths the data, and rejects erroneous reports. An automatic data input is soon to be added to the system.

The forecasts are now issued to the National Weather Analysis Center in Suitland, to the Air Weather Service, and to Navy Aerology.

A comparison of the baroclinic machine forecasts with those prepared by the NWAC forecasters indicates that for 24-hour forecasts for the 500 mb. level there is no difference in skill.

It has been found that the accuracy of the machine forecasts decreases with decreasing elevation. At 900 mb., for example, the forecasts show relatively low skill. The accuracy of the forecasts also decreases with the length of the forecast period. The forecasts show greatest skill over those areas where the data are most plentiful (the United States) and where the western boundary conditions are best known (the North Atlantic).

*This paper was presented at a meeting of the Section on March 27, 1956.

At the present time, the forecasts for 500 mb. prepared by the three-level model are no better than the barotropic forecasts.

Major sources of error are the effects of boundary conditions; inadequate data, especially over the Pacific Ocean; the effects of mountains; truncation errors resulting from the use of finite differences; errors of linearization, such as the use of a constant static stability factor; and the use of the geostrophic approximation in low latitudes. The last two are responsible for the tendency of the computer to overpredict anticyclogenesis.

Steps are being taken to remove all these sources of error, and recent experiments with new (nongeostrophic) models indicate that the forecasts will be improved.

SECTION OF ANTHROPOLOGY

THE ANTECEDENTS OF ZUNI CULTURE*

By Richard B. Woodbury

Department of Anthropology, Columbia University, New York, N. Y.

It is more than 15 years since Elsie Clews Parsons wrote of the unfortunate gap that still exists between ethnology and archeology in the Southwest, and mentioned, among special problems crying for attention, that "Between Zuñi (New Mexico) and Acoma (New Mexico) there are many still-unexcavated sites that should yield data about early Keres-Zuñi contacts.... From the point of view of tribal acculturation it should be one of the most valuable exploratory places in the Southwest" (Parsons, 1940; pp. 216-217). I am sure that Parsons would be pleased to know that during the last 10 years this 75-mile gap in our knowledge has been the scene of both extensive archeological reconnaissance and excavation. During the 13th and 14th centuries, it now appears, the Zunis and Acomas were much closer neighbors, with the barren lava flows that stretch south from Mt. Taylor and the forests of the Zuni Mountains probably forming a natural boundary zone, little populated but easily traversed.

The Acoma area has been investigated by A. E. Dittert, Jr. and R. J. Ruppé, Jr. and, although full reports on their work have not yet been completed, the results have been summarized in three articles in *El Palacio* (Dittert and Ruppé, 1951; Ruppé and Dittert, 1952, 1953). Besides finding and investigating many earlier sites in the region west of Acoma, Dittert and Ruppé discovered a large Pueblo III site (LP2:24-D) about 15 miles west of Acoma on a mesa top, well fortified and, in both architecture and pottery, closely resembling early Acoma. In addition they were permitted to make excavations within the modern pueblo of Acoma itself. They discovered that the site was settled about 1100 to 1200 by people already living in the area. With the abandonment of villages to the west, including LP2:24-D, the Acomas were concentrated in the town that survives to the present. Thus, prior to about 1400, the Acoma settlement was approximately 15 or 20 miles nearer to Zuni than today.

Archeological work in the Zuni area dates back to 1888, when the Hemenway Expedition shifted operations to that area from southern Arizona and briefly investigated a few sites (Fewkes, 1891). From 1917 to 1920 F. W. Hodge excavated extensively at Hawikuh and Kechipawan, two of the six Zuni villages that the Spanish inappropriately called the Seven Cities of Cibola when they entered the Southwest in 1540. In 1915

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on March 26, 1956.

and 1916 N. C. Nelson, Leslie Spier, and A. L. Kroeber located and described a large number of ruins near Zuni and extending eastward about 30 miles to El Morro (also known as Inscription Rock). Finally, in the 1930's, Roberts carried out important excavations in sites covering a long span of time prior to Pueblo IV.

My own work in the Zuni area began in 1953 and is still in progress. Definitive results cannot yet be presented, but the picture can at least be roughed in. The work began with reconnaissance of the area, in which I and my collaborators relied heavily on Spier's excellent work (Spier, 1917), and was then concentrated on a single site, a small portion of which we excavated. Atsinna Ruin, as we named it at the suggestion of our Zuni workmen, appeared from sherds on its surface to be older than the historic Zuni villages such as Kechipawan and Hawikuh, yet recent enough to overlap with their occupation. The National Park Service at the El Morro National Monument, N. Mex., granted permission for excavation and cooperated very generously in the work. During 1954 a dozen rooms were dug, the largest of them, a round kiva, being completed during 1955. Test trenches in rubbish accumulations in various parts of the site totaled nearly 100 meters in length, and provided a stratigraphic sequence of sherds for the occupation span of the site.

Atsinna Ruin is situated on the northern part of the mesa, the northern tip of which forms the conspicuous landmark of El Morro, where early Spanish explorers and later emigrants and military surveyors left their names carved on the sheer sandstone cliff. The deep, shaded pool at the foot of the cliff, fed only by run-off but almost never failing, is the reason for the relatively recent popularity of the spot, and it doubtless attracted Indian villagers many centuries earlier as well. There were scattered one-family settlements at the foot of the mesa and out into the valley in the Pueblo I and II periods (approximately A.D. 700 to 1100), and possibly on top of the mesa also. It was during Pueblo III times, about 1100 to 1300, however, that the population of the Zuni region began to concentrate in large pueblos, most of them on mesa tops. This movement took place in many other parts of the Southwest at about the same time, and has been variously explained. It was a gradual trend, and not a sudden shift, just as there has been a trend during the past century for pueblos to expand into a more scattered type of settlement, with movements down from mesa tops and out into small, summertime farming colonies. The change in settlement patterns in the 12th and 13th centuries appears to have had a variety of causes, with the incursion of Athabascan or other nomads playing at the most a minor role. Hostility and outright warfare between neighboring pueblos may have been a factor, and it seems especially probable that the increasing complexity of the social and ritual organization of an expanding population made such a village pattern desirable and that, at the same time, an increasingly

productive economy with a surplus to be diverted to the support of various specialists made it possible.

The first rooms built at Atsinna are now covered by the occupational refuse of succeeding generations. Nevertheless, at the bottom of our deepest stratigraphic trench, approximately in the center of the large inclosed plaza of the pueblo, we were fortunate enough to find the lower courses of some of these first walls resting on bedrock and overlaid by two meters of rubbish and later construction. The orientation of these walls was markedly different from that of the later town, but they were built in the same fashion of coursed masonry, using carefully shaped sandstone blocks. The associated pottery suggests a date early in the 13th century, and further study will probably verify and refine this estimate. During the next century and a half the village grew rapidly and, probably in a single burst of activity, was laid out anew with massive outer walls and a large central plaza.

At the time of its abandonment in the latter part of the 14th century, Atsinna was a compactly built town of 500 to 1000 rooms, forming an approximate rectangle about 65 by 100 meters. Rooms were ranged three to five deep around the central plaza, on which they gradually encroached as the population expanded. Scarcely any rooms were built outside the main wall, but inside it houses rose to a height of two and three stories, terraced down to a single story along the plaza. No doors or windows broke the outer wall, at least in its lower portions, unless possibly on the southern side, where two narrow gaps in the ruined walls may indicate either a passage at ground level or vandalism and treasure hunting prior to federal protection of the ruin. Although the mesa on which Atsinna stands has a sheer northern face, the less precipitous southerly portion is easily climbed, and mere location could not have ensured protection from raiders. With its elevated position, however, an unseen approach would be difficult, and the ground plan and manner of construction suggest an attempt at defensibility.

Although there are no nearby springs, the village had an unusually good water supply. The natural pool at the base of the mesa has been mentioned. A short, steep trail formerly led down to it, with toe and finger holes picked in the sandstone in the steeper places. In addition there are many small basins and pools in the bare rock of the mesa top, some of them artificially enlarged. These depressions would fill at every thunderstorm, and from them many storage jars of good water could easily have been secured. Also on the mesa top, just south of the ruin, are three faintly visible circular depressions. We trenched one of them and it proved to be a reservoir about 7.5 meters in diameter and 1 meter deep, dug into the sandstone of the mesa. The other two appeared to have been similar and, when filled by run-off, would have held water for several days in summer and for many weeks in winter. Even if not

suitable for consumption, which seems unlikely, the water from these tanks would have helped supply the quantities of water for mortar and plaster required by incessant repairs and building.

Although the rooms we cleared were only a small sample for such a sizable pueblo, they sufficed to reveal a number of important things about the site. In the first place, both a rectangular and a circular kiva were found. Both were surrounded by domestic rooms and, although entered by ladders through their roofs, they were not actually below ground. The rectangular kiva conformed in general to the Zuni kiva excavated at Hawikuh by Hodge (1937 and 1939), which dates from about 1400 and continued in use into the period of Spanish contact. The Atsinna kiva has a broad southern bench with a ventilator passage running under it, narrower benches on the east and west sides, and a central fireplace with a large U-shaped deflector. On the floor were found fragments of a stone slab with a large oval hole in the center, carefully ground smooth, which may have framed the ventilator opening.

The round kiva, with a diameter of about nine meters, had a broad platform across the south side, with the ventilator passage through it, and a narrow bench around the rest of the circumference. The roof was supported by six large masonry-inclosed posts, three against the east wall and three against the west. Either side of the fireplace we found indications of a floor pit, less than a meter square, stone bordered, and probably originally covered with poles to serve as a resonator or foot drum. Round kivas have not been known at Zuni in historic times, but they are characteristic of the Keresan pueblos of the Rio Grande and they have a long history in the San Juan drainage. In the Zuni area, round kivas occurred in a Pueblo III village about 20 miles northwest of Atsinna in Nutria Canyon (Roberts, 1932) and in a small site near Hawikuh (Hodge, 1923). In both instances, kiva details and the associated pottery showed strong similarities to the Chaco Canyon area, about 75 miles to the north. The Zuni drainage, at the time of these Chacoan Pueblo III kivas, was near the southern periphery of a large region of San Juan Anasazi culture, of which Chaco Canyon was a major center of population concentration and cultural elaboration. When most of that area was abandoned in the 12th and 13th centuries the Zuni region remained continuously occupied and probably received some minor population accretions from the deserted area. At that time, however, the Zuni drainage came increasingly under Western Pueblo influence, from eastern and central Arizona, an influence that extended through the Zuni towns to the Acoma area and eventually to the Rio Grande. The round kiva at Atsinna, which sherd analysis can probably date in the 14th century, would thus represent one of the last expressions in the Zuni area of a northern tradition in religious architecture that can be traced back in the San Juan drainage through the earliest round kivas to their pit house prototypes of the fifth and sixth centuries (Brew, 1946).

Some of the domestic rooms we cleared were remarkable for certain elaborate details that are more often found in kivas: floors paved with carefully fitted and smoothed stone slabs, benches along the south or east walls, putative ventilators and, in one instance, geometric designs painted in several colors on a plastered wall. Burials were scarce, as is generally the case in Pueblo IV ruins. The two adult skeletons found were in rooms that had been abandoned for domestic purposes and, after the burials had been made, had been sealed off from the adjoining, still-occupied rooms.

It is from potsherds that the archeologist usually derives his most precise data on cultural affiliations, as well as his best chronological data. Although our ceramic analysis is not yet complete, it appears that Reed's general reappraisal of Zuni ceramic history (Reed, 1955) will be borne out in most details, thus requiring some revisions in the scheme of classification presented by the Museum of Northern Arizona, Flagstaff, Ariz., a number of years earlier (Colton and Hargrave, 1937). Particular interest attaches to the classification and sequence of Zuni pottery types in late Pueblo III and early Pueblo IV because of the local development of glaze decoration and its spread to the Rio Grande pueblos, where it underwent a further development and then gradual artistic degeneration after it was given up by the Zunis as a decorative technique. In the 15th century the Zunis also created a vigorous but comparatively crude imitation of the elegant polychrome pottery of the Hopis. Details of the pottery from Atsinna cannot be discussed here, but one important conclusion can be stated, based on the preliminary study of sherds from numerous sites in the area, a conclusion foreshadowed by Spier in his 1917 work. The desertion of Atsinna does not represent any significant break in the cultural continuity of the Zuni area, but merely removal to another village site. There are nearly a dozen other ruins approximately contemporary with Atsinna, scattered along both sides of the valley that stretches westward to Zuni, and it appears that during the latter part of the 14th century all of them were abandoned. It is probable that their occupants moved to the six large villages that make up the early historic Zuni group, since these villages had their major growth in the 15th to 17th centuries. There is no indication at Atsinna of hasty departure. The rooms we dug had been stripped of every valuable or useful object, and several of them had been used as rubbish dumps. This suggests a gradually dwindling population, the villagers no longer building new rooms and no longer carrying trash to the extramural dumps, but living on in the dying town and, year by year, growing fewer. Pecos and Oraibi show us much the same thing in historic times.

A reason for this contraction is not immediately apparent. It parallels the withdrawal of the ancestral Acomas to the eastward at about the

same time. As a result the distance between the towns of the Zunis and Acomas was about 75 miles instead of 25 miles. The fact that much of the intervening area is not farmed today or is farmed with only meager returns, might be taken as evidence of lessened rainfall. Such a climatic change cannot be demonstrated and, from what we know of climatic trends, it is a shaky explanation. The erosional cycle that cut arroyos in the broad valleys and thus lowered the water tables of many Southwestern localities in the 13th century may have finally had an influence here (Reed, 1944), but arroyo cutting proceeds upstream and would thus be expected to make the western, downstream part of the Zuni drainage nonarrable before it affected the El Morro area. It is possible that a decline in population, the reason for which we have yet to discover, was the impetus for abandoning outlying settlements and withdrawing into fewer but larger towns. Whatever the cause, however, the result was the consolidation of separate groups, again and again, with the consequent necessity for the creation of (or the strengthening of already existing) social mechanisms by which social, economic, and ceremonial affairs could be smoothly readjusted and continued. Eggan has commented that "The social organization of Zuni is perhaps the most complex to be found in the western Pueblos; the social integration achieved here is both strong and successful" (Eggan, 1950 : 210). It may well be that this complexity derives in part from the disparate antecedents of the Zunis, slowly consolidated over the centuries from formerly independent towns and doubtlessly incorporating from time to time bands of migrants from other areas also.

Two postscripts should be added to this discussion. First, we have not mentioned linguistic evidence, which is frequently a valuable aid to historical reconstruction. For Zuni, however, linguistic evidence offers little help for the period we have dealt with, as the Zuni language is not closely related to any other known language, and distant and uncertain connections with Aztec-Tanoan (Sapir, 1929) or with Tarascan (M. Swadesh, unpublished data) refer to a period at least two or three millenia back. Second, Zuni burial practices deserve careful study and may provide important clues to historical origins. The Spanish chroniclers report cremation as a Zuni practice, and Hodge found cremations in the late prehistoric period. Since this is an unusual burial practice for the pueblo area, and is characteristic of the Hohokam of southern Arizona, influence from the south appears to have taken place. Recently excavated cremations from the Point of Pines area of Arizona will also need to be considered for their bearing on the problem of Zuni antecedents.

In conclusion, there seems little doubt that Zuni civilization has been subjected to a great variety of contacts and influences through the past six or seven centuries, particularly from the Chaco area in Pueblo III times, and probably, subsequently, from the south and east. The Zuni

contacts with both the Hopi and the Acoma groups have been close, and through the latter it has influenced the Rio Grande. That Zuni has preserved its distinctiveness and independence testifies to the vigor of its conservative tradition. That the social and ceremonial systems were complex, and even confusing, is hardly surprising in view of their repeated adjustment to consolidation and amalgamation of hitherto separate groups.

Acknowledgments

This work, from its beginning, has had the generous support of the Anthropology Department of Columbia University, New York, N. Y., and the support of grants in 1954 and 1955 by the Columbia University Council for Research in the Social Sciences and, in 1956, by the Wenner-Gren Foundation for Anthropological Research, New York, N. Y. Throughout the work I have had the invaluable collaboration of my wife, Nathalie F. S. Woodbury, of Barnard College, New York, N. Y. Four Columbia University graduate students have also participated in this work: Arthur Fields, Richard Forbis, Timothy O'Leary, and Charles W. Ward.

References

BREW, J. O. 1946. Archaeology of Alkali Ridge, Southeastern Utah. Pap. Peabody Mus. 21.

COLTON, H. S. & L. L. HARGRAVE. 1937. Handbook of Northern Arizona Pottery Wares. Mus. N. Ariz. Bull. **11**: 113-117.

DITTERT, A. E. & R. J. RUPPÉ. 1951. The archaeology of Cebolleta Mesa. El Palacio. **58**(4): 116-129.

EGGAN, F. 1950. Social Organization of the Western Pueblos. Univ. of Chicago Press, Chicago, Ill. : 210.

FEWKES, J. W. 1891. Reconnaissance of ruins in or near the Zuni Reservation. J. Am. Ethnol. Archaeol. **1**: 94-132.

HODGE, F. W. 1923. Circular kivas near Hawikuh. Mus. Am. Indian Contrib. 7(1): 1-37.

HODGE, F. W. 1937. History of Hawikuh. Southwest Mus. Los Angeles.

HODGE, F. W. 1939. A square kiva at Hawikuh. *In* So Live the Works of Men (ed. by D. D. Brand & F. E. Harvey). Univ. of New Mexico Press. Albuquerque, N. Mex. : 195-214.

PARSONS, E. C. 1940. Relations between ethnology and archaeology in the Southwest. Am. Antiq. **5**(3): 214-220.

REED, E. K. 1944. The abandonment of the San Juan region. El Palacio. **51**(4): 61-74.

REED, E. K. 1955. Painted pottery and Zuñi history. Southwestern J. Anth. 11(2): 178-193.

ROBERTS, F. H. H. 1932. The Village of the Great Kivas, New Mexico. Bur. Am. Ethnol. Bull. 111.

RUPPÉ, R. J. & A. E. DITTERT. 1952. The archaeology of Cebolleta Mesa and Acoma Pueblo. El Palacio. **59**(7): 191-217.

RUPPÉ, R. J. & A. E. DITTERT. 1953. Acoma archaeology. El Palacio. **60**(7): 259-273.

SAPIR, E. 1929. Central and North American Languages. Ency. Brit. 14th Ed. 5: 138-141.

SPIER, L. 1917. An outline for a chronology of Zuñi ruins. Am. Mus. Nat. Hist. Anth. Pap. **18**(3): 207-331.

NOTICE TO ACTIVE MEMBERS

In accordance with the Bylaws of the Academy, Article VII, Section 7, the Nominating Committee of the Council is inserting in this April issue a notice to Active Members, quoting Article IV, Section 1 of the Bylaws.

Article IV, Section 1 reads as follows:

"*Suggestions by the Members for Nominations.* Suggestions for nominations of Officers of the Academy and Members of the Council, with the exception of Sectional Chairmen, may be sent in writing by any Active Member of the Academy, addressed to the Nominating Committee of the Academy, 2 East Sixty-third Street, New York, N.Y., with the name of the proposer. To be considered, such suggestions must be received not later than September 1 of each year."

CONFERENCES HELD

SECTION OF BIOLOGY

NATURAL RESISTANCE TO INFECTIONS

Thursday, March 1 and
Friday, March 2, 1956

Conference Chairman: Walsh McDermott
Cornell Medical College, New York, N. Y.

THURSDAY, MARCH 1, 1956

Session Chairman: Hilary Koprowski
Lederle Laboratories Division
American Cyanamid Company, Pearl River, N. Y.

9:00 A.M. –

Greetings from the Academy – Hilary Koprowski, Chairman, Section of Biology, New York Academy of Sciences, New York City; Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

"Present Status of Knowledge of the Properdin System" – L. Pillemer, Institute for Pathology, Western Reserve Medical School, Cleveland, Ohio.

"Bactericidal Activities of the Properdin System" – R. J. Wedgewood, Western Reserve Medical School, Cleveland, Ohio, and A. C. Wardlaw, Wright-Fleming Institute for Microbiology, St. Mary's Hospital, London, England.

"Inactivation of Virus by the Properdin System" – H. S. Ginsberg, Western Reserve Medical School, Cleveland, Ohio.

"The Relationship of the Toxoplasma Antibody Activator to the Serum Properdin System" – H. Feldman, College of Medicine, Upstate Medical Center, Syracuse, N. Y., and L. Pillemer, Institute for Pathology, Western Reserve Medical School, Cleveland, Ohio.

"Properdin Levels in Infectious and Non-Infectious Diseases" – C. F. Hinz, Jr., Institute for Pathology, Western Reserve Medical School, Cleveland, Ohio.

Session Chairman: A. A. Miles
Lister Institute of Preventive Medicine
London, England

2:00 P.M. –

"The Properdin System in Relation to Fatal Bacteremia Following Total

Body Irradiation of Laboratory Animals" – O. A. Ross, Institute for Pathology, Western Reserve Medical School, Cleveland, Ohio.

"The Effect of Irradiation on Natural Resistance to Infection" – C. P. Miller, University of Chicago, Chicago, Ill.

"Increase in Resistance Following Administration of Bacterial Lipopolysaccharides" – M. Landy, Army Medical Service, Graduate School, Washington, D. C.

"Factors Influencing Natural Resistance to Bacterial Infections" – R. Rowley, Wright-Fleming Institute for Microbiology, St. Mary's Hospital, London, England.

"The Effect of Various Substances on Resistance to Experimental Infections" – J. S. Kiser, H. Lindh and G. C. de Mello, American Cyanamid Company, Pearl River, N. Y.

FRIDAY, MARCH 2, 1956

Session Chairman: Rene Dubos
Rockefeller Institute for Medical Research
New York, N. Y.

9:00 A.M. –

"Effect of Dextrans and Levans on the Inflammatory Process" – S. Hestrin, Hadassah Medical School, Hebrew University, Jerusalem, Israel.

"Host Resistance to Bacteria in Traumatic Shock" – Jacob Fine, Beth Israel Hospital, Boston, Mass.

"Nutritional and Genetic Factors in the Natural Resistance of Mice to Salmonella Infections" – Howard Schneider, Rockefeller Institute for Medical Research, New York, N. Y.

"Cellular Products Affecting the Establishment of Bacteria of Different Virulence" – Werner Braun, Institute of Microbiology, Rutgers University, New Brunswick, N. J.

"Non-Specific Defence Reactions in Bacterial Infections" – A. A. Miles, Lister Institute of Preventive Medicine, London, England.

Session Chairman: J. S. Kiser
American Cyanamid Company, Pearl River, N. Y.

2:00 P.M. –

"Effect of Components of the Tricarboxylic Acid Cycle on Bacterial Infections" – L. J. Berry, Bryn Mawr College, Bryn Mawr, Pa.

"Biochemical Studies on the Bactericidal Power of Phagocytic Cells" J. G. Hirsch, Rockefeller Institute for Medical Research, New York, N. Y.

"Phagocytosis Promoting Factors of Plasma and Serum" – J. L. Tullis, Harvard Medical School, Boston, Mass.

"Serum Bactericidins Active Against Gram-negative Bacteria" – Q. N. Myrvick, University of Virginia School of Medicine, Charlottesville, Va.

Summary of Conference – Walsh McDermott, Cornell Medical College, New York, N. Y.

EXPERIMENTAL METHODS FOR THE EVALUATION OF DRUGS IN VARIOUS DISEASE STATES

Thursday, March 8 and
Friday, March 9, 1956

Conference Chairman: Bradford N. Craver
Squibb Institute for Medical Research, New Brunswick, N. J.

THURSDAY, MARCH 8, 1956

COLLAGEN DISEASES

Session Chairman: William E. Ehrich
University of Pennsylvania, Philadelphia, Pa.

9:30 A.M. –
Greetings from the Academy – Hilary Koprowski, Chairman, Section of Biology, New York Academy of Sciences, New York City; Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

The Conference and Its Aims – Bradford N. Craver, Squibb Institute for Medical Research, New Brunswick, N. J.

"Assay of Antiphlogistic and Anti-anaphylactic Effects" – Thomas F. Dougherty, University of Utah School of Medicine, Salt Lake City, Utah.

"Assay of Drugs on Connective Tissue Permeability" – Joseph Seifter, Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.

"Screening by Means of Eggwhite Anaphylaxis, Granuloma Pouch, and Periarteritis" – Hans Selye, University of Montreal, Montreal, Quebec, Canada.

ANGINA PECTORIS

Session Chairman: Donald E. Gregg
Walter Reed Army Medical Center, Washington, D. C.

2:00 P.M. –
"The Clinical Aspects for the Appraisal of Drugs Used for the Treatment of the Anginal Syndrome" – Robert C. Batterman, New York Medical College, New York, N. Y.

"Determinants of Coronary Flow. Design of Proper Experiments to Investigate Clinical Angina" – Louis N. Katz, Michael Reese Hospital, Chicago, Ill.

"The Neurochormonal Factors in the Origin and Treatment of Angina Pectoris" – Wilhelm Raab, University of Vermont College of Medicine, Burlington, Vt.

"Evaluation of Drugs Used in the Treatment of Angina Pectoris by Means of Exercise – Electrocardiographic Tests" – Henry I. Russek, New York Medical College, New York, N. Y.

CARDIAC ARRHYTHMIAS

Session Chairman: Gordon K. Moe
State University of New York, Syracuse. N. Y.

"Arrhythmias Following Experimental Coronary Occlusion and Their Response to Drugs" – Byron B. Clark, Tufts College Medical School, Boston, Mass.

"Screening of Drugs for Anti-arrhythmia Activity" – Charles H. Ellis, Wellcome Research Laboratories, Tuckahoe, N. Y.

"Relation Between Atrial and Ventricular Anti-arrhythmic Assay Methods: Rationale for a Screening Program" – Martin M. Winbury, Schering Corporation, Bloomfield, N. J.

"Correlation Between Pharmacological Test and Clinical Test" Luther L. Terry, National Institutes of Health, Bethesda, Md.

FRIDAY, MARCH 9, 1956

ATHEROSCLEROSIS

Session Chairman: Howard A. Eder
State University of New York, College of Medicine
Brooklyn, N. Y.

9:30 A.M. –
"Inhibitors of Cholesterol Synthesis and the Problem of Hypercholesterolemia" – Daniel Steinberg and Donald S. Frederickson, National Heart Institute, Bethesda, Md.

"Lipoproteins as a Screening Measure for Evaluation of the Evolution of Arteriosclerosis" – John W. Gofman, University of California, Berkeley, Calif.

"Experiences in Assessment of Estrogen Anti-atherogenesis in Chick, Rabbit and Man" – Jeremiah Stamler, Michael Reese Hospital, Chicago, Ill.

"The Prediction of Atherosclerotic Complications in Hypertensive Patients" – Arthur Corcoran, Cleveland Clinic, Cleveland, Ohio.

DISORDERS OF THE CENTRAL NERVOUS SYSTEM

Session Chairman: James E. P. Toman
Chicago Medical School, Chicago, Ill.

2:00 P.M. –
"A Comparative Approach to the Evaluation of Drug Effects Upon Affective Behavior" – Joseph V. Brady, Walter Reed Army Medical Center, Washington, D. C.

"Use of Intracerebral Electrodes to Evaluate Drugs which Act on the Central Nervous System" – Jose M. R. Delgado, Yale School of Medicine, New Haven, Conn.

"The Experimental Evaluation of Sedative Agents in Animals" – Robert Lim, Miles-Ames Research Laboratories, Elkhart, Ind.

"Chemical Convulsant Indices" – Carl Pfeiffer, Emory University, Emory, Ga.

"Experimental Assay of Anti-tremor Agents" – Vernon G. Vernier, Walter Reed Army Medical Center, Washington, D. C.

"Correlation Between Animal Testing Procedures and Clinical Effectiveness of Centrally Acting Muscle Relaxants of the Mephenesin Type" – Edward F. Domino, University of Michigan, Ann Arbor, Mich.

SECTION OF PHYSICS AND CHEMISTRY

UNSTABLE CHEMICAL SPECIES (FREE RADICALS, IONS, AND EXCITED MOLECULES)

Thursday, March 15 and
Friday, March 16, 1956

Conference Co-Chairmen

Thomas Freund, New York University, New York, N. Y.

Henry C. Thacher, Jr., Aeronautical Research Laboratory, Wright Air Development Center, Ohio

THURSDAY, MARCH 15, 1956

Session Chairman: Brian H. Mason
American Museum of Natural History, New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Frank C. Collins, Chairman, Section of Physics and Chemistry, New York Academy of Sciences. New York City; Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Introductory Remarks – Henry C. Thacher, Jr., Chemistry Research Branch, Aeronautical Research Laboratory, Wright Air Development Center, Ohio.

"The Occurrence of Unstable Species in the Atmosphere" – Gerard P. Kuiper, Yerkes Observatory, University of Chicago, Chicago, Ill.

"The Production of Unstable Species in Shock Waves" – D. F. Hornig, Brown University, Providence, R. I.

"Forces between Unstable Species" – J. C. Hirschfelder, University of Wisconsin, Madison, Wis.

Session Chairman: Henry C. Thacher, Jr.
Aeronautical Research Laboratory, Wright Air Development Center, Ohio

2:00 P.M. –

"Mass Spectroscopy of Free Radicals" – F. P. Lossing, National Research Council, Ottawa, Canada.

"Mass Spectroscopy of Gases Subjected to Electrical Discharge" – H. I. Schiff, McGill University, Montreal, Canada.

"Paramagnetic Resonance of Free Radicals" – George K. Fraenkel, Columbia University, New York, N. Y.

"Absorption Spectra of Free Radicals" – D. A. Ramsay, National Research Council, Ottawa, Canada.

FRIDAY, MARCH 16, 1956

Session Chairman: Thomas Freund
New York University, New York, N. Y.

9:00 A.M. –

"Photochemical Reactions Leading to Unstable Species" – H. Austin Taylor, New York University, New York, N. Y.

"Kinetics of Energy Exchange in Molecules" – M. Boudart, Princeton University, Princeton, N. J.

"Stabilization of Free Radicals at Low Temperatures" – H. P. Broida, National Bureau of Standards, Washington, D. C.

"Sputtering of Metals by Low Energy Mercury Ions" – Gottfried Wehner, General Mills, Inc., Detroit, Mich.

Session Chairman: Harold Blatt
Queens College, Flushing, L.I., N. Y.

2:00 P.M. –

"Reactions of Ionic Beams with Crystalline Surfaces" – Walter J. Moore, Cormac O'Briain and Amelie Lindner, Indiana University, Bloomington, Ind.

"The Role of Free Radicals in Organic Reaction Mechanisms" – Cheves Walling, Columbia University, New York, N. Y.

"The Role of Unstable Species in High Temperature Kinetics" – John L. Margrave, University of Wisconsin, Madison, Wis.

"The Role of Free Radical Reactions in Biological Oxidations" – E. S. Guzman Barron, University of Chicago, Chicago, Ill.

NEW MEMBERS

Elected March 22, 1956

SUSTAINING MEMBERSHIP

Haas, Ward J., Ph.D., Biochemistry. New Products Co-ordinator, Chas. Pfizer & Co., Inc., Brooklyn, N.Y.

Johnstone, Allan Mackenzie, M.D., Medicine. Private Practice, Wananish, N.C.

Nakayama, Komei, M.D., General Surgery. Professor, Surgery, Chiba University, Chiba, Japan.

Nonas, Gerson, M.D., Endocrinology. Assistant Physician, Flower and Fifth Avenue Hospital, New York, N.Y.

Orfitelli, O. P., M.D., Medicine. Private Practice, Hartford, Conn.

ACTIVE MEMBERSHIP

Abrahamson, W. Vincent, B.A., Pharmaceutical Manufacturing. Vice President, General Manager, Ortho Pharmaceutical (Canada) Ltd., Toronto, Ont., Canada.

Acevedo, Dario, M.D., Physiology. Faculty of Science Dean, Universidad de San Marcos, Lima, Peru.

Aguirre, Alejandro, M.D., Research, Chemotherapy of Cancer. Associate Professor, Pediatrics, National University of Mexico, Mexico City, Mexico.

Alving, Alf Sven, M.D., Medical Research. Professor, Medicine, Billings Hospital, Chicago, Ill.

Andreassian, M.G., M.D., Biology, Psychology. Clinical Instructor, Orthopedics, Southwestern Medical School, Texas University, Dallas, Texas.

Andresen, Richard H., M.D., Experimental Pathology. Clinical Assistant Professor, Obstetrics, Gynecology, University of Illinois, Chicago, Ill.

Angelino, Henry, Ph.D., Psychology. Associate Professor, Education, University of Oklahoma, Norman, Okla.

Armistead, Lewis Moore, M.A., Psychology. Assistant Director, Division of Clinical and Medical Psychology, University of Virginia Hospital, Charlottesville, Va.

Austin, Edward B., LL.B., Pharmaceuticals. President, Thos. Leeming & Co., Inc., New York, N.Y.

Basmajian, John V., M.D., Anatomy. Associate Professor, University of Toronto, Toronto, Ont., Canada.

Bauer, Virginia E., B.A., Analytical Chemistry. Chemist, Army Chemical Center, Md.

Beck, Donald, B.A., Zoology. Biochem. Research Assistant, Chas. Pfizer & Co., Booklyn, N.Y.

Bell, E. John, D.Sc., Virus Diseases. Senior Scientist, U.S. Public Health Service, Hamilton, Mont.

Berg, Gerald, Ph.D., Virology. Virologist, U.S. Public Health Service, Cincinnati, Ohio.

Berle, Beatrice Bishop, M.D., Biology. Private Practice, New York, N.Y.

Berlin, Byron Sanford, M.D., Immunology. Research Associate, School of Public Health, University of Michigan, Ann Arbor, Mich.

Berlin, Irving N., M.D., Psychiatry. Instructor, University of California, San Francisco, Calif.

Beron, Peter, Ph.D., Psychology. Supervising Psychotherapist, Lafargue Clinic, New York, N.Y.

Bilenker, Eugene N., Ph.D., Food Technology. Livingston, N.J.

Bliss, Eleanor A., D.Sc., Bacteriology. Professor, Biology, Bryn Mawr College, Bryn Mawr, Pa.

Bonk, Johan P., M.D., Radiology. Radiologist, 72nd Tactical Hospital, Ramsey A.F.B., Puerto Rico.

Bower, John J., LL.B., Psychology. Partner, Bower and Heffernan Esqs., New York, N.Y.

Box-Hutchinson, Louise A., M.D., Internal Medicine. Instructor, Tulane University, New Orleans, La.
Bratton, Andrew C., Jr., M.D.. Pharmacology. Director, Pharmacological Research, Parke, Davis & Company, Detroit, Mich.
Breiner, Sander James, M.D., Psychiatry. Staff Psychiatrist, Ypsilanti State Hospital, Ypsilanti, Mich.
Bronk, Theodore T., M.D., Medicine. Pathologist, Director, Laboratories, Mt. St. Mary's Hospital, Niagara Falls, N.Y.
Bryan, W. Ray, Ph.D., Cancer Research. Biologist, National Cancer Institute, National Institutes of Health, Bethesda, Md.
Bryant, James Marion, M.D., Cardiovascular Diseases. Associate Professor, Medicine, New York University Post Graduate Medical School, New York, N.Y.
Busada, Samuel George, Chemical Engineering. Research Laboratory Technician, North Bergen, N.J.
Butterworth, J. Scott, M.D., Cardiology. Associate Professor, Medicine, New York University Post Graduate Medical School, New York, N.Y.
Calise, Vincent J., M.E., Process Water. Research and Sales Development Manager, Long Beach, N.Y.
Carlson, Robert H., M.D., Internal Medicine. Resident Physician, San Francisco Veterans Administration Hospital, San Francisco, Calif.
Carter, Thomas Milton, Ph.D., Educational Psychology. Head, Department, Education, Psychology, Albion College, Albion, Mich.
Cervantes, Armando Ramirez, M.D., Medicine. Resident, Neurosurgery, Bellevue Hospital, New York, N.Y.
Chamlin, Max, M.D., Ophthalmology. Associate Professor, Surgery, Albert Einstein College of Medicine, New York, N.Y.
Charles, Martin L., M.D., Calcium Metabolism. Research Fellow, Metabolic Unit, Neoplastic Division, Montefiore Hospital, New York, N.Y.
Chase, Merrill W., Ph.D., Experimental Immunology. Associate Member, The Rockefeller Institute for Medical Research, New York, N.Y.
Cheng, Edmund Wei-Kuang, Ph.D., Physiology. Assistant Professor, Iowa Agricultural Experiment Station, Ames, Iowa.
Clark, William B., M.D., Ophthalmology. Professor, Tulane University, New Orleans, La.
Clark, William Clarence, Biology. Indiana University, Indianapolis, Ind.
Cleaver, C. Perry, M.D., Medicine. Private Practice, Catawissa, Pa.
Cléroux, P. Alfred, M.D., Internal Medicine. Assistant, Medicine, Hotel-Dieu Hospital, Montreal, P.Q., Canada.
Clevans, Mark, Psychology. Psychoanalyst, New York, N.Y.
Cochran, J. Lawrence, M.D., Psychology. Director, Cochran Clinic, Carroll, Iowa.
Coe, H. S., M.D., Medicine. Private Practice, Kenmore, N.Y.
Comer, Edward O. B., M.D., Proteins, Immunity. Staff Physician, Veterans Hospital, New Orleans, La.
Cooper, Gerald R., M.D., Experimental Medicine. Chief, Hematology, Communicable Disease Center, Chamblee, Ga.
Cordaro, Joseph V., M.D., Electrocardiography. Resident, Internal Medicine, Veterans Administration Hospital, Brooklyn, N.Y.
Corrado, Michael, M.D., Medicine. Georgetown University Hospital, Washington, D.C.
Cort, Winifred M., Ph.D., Biochemical Microbiology. Group Leader, Fermentation and Enzymes Fundamental Division, National Dairy Research Laboratories Inc., Oakdale, N.Y.
Corwin, William, M.D., Psychiatry. Instructor, University of Miami, Coral Gables, Fla.
Crawshaw, Ralph, M.D., Psychiatry. The Menninger Foundation, Topeka, Kans.
Davis, David, Ph.D., Plant Pathology. Merck & Co., Inc., Rahway, N.J.
Dedrick, Jean W., Ph.D., Medical Microbiology. Director, Laboratory, Communicable Disease Unit, Los Angeles County Hospital, Los Angeles, Calif.
Denber, Herman C. B., M.D., Psychiatry. Director, Psychiatric Research, Manhattan State Hospital, New York, N.Y.
Dessel, B.H., M.D., Hematology. Sectional Chief, Hematology, Veterans Administration Center, Wood, Wis.

Devins, Edward A., M.D., Animal Psychology. Private Practice, Kansas City, Mo.

Dobias, Bohdan, M.D., Pediatrics. Assistant, Dermatology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

East, W. Marshall, M.D., Plastic Reconstructive Surgery. Assistant Resident Surgeon, St. Vincents Hospital, New York, N.Y.

Eckerstrom, Sten, M.D., Internal Medicine. Head Physician, Vasa Hospital, University of Gothenburg, Gothenburg, Sweden.

Edsall, Geoffrey, M.D., Immunology. Director, Immunology Division, Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

Edwards, Robert E., Ph.D., Psychophysiology. Assistant Professor, Psychology, Rensselaer Polytechnic Institute, Troy, N.Y.

Einsel, Thomas H., M.D., Medicine. Surgical Staff, Woman's Hospital, Cleveland, Ohio.

Eisner, Hans E., Ph.D., Biology. Vice President, Norvel Labor Inc., Tuckahoe, N.Y.

Elliott, Floyd M., M.D., Medicine. Private Practice, Ada, Ohio.

Ellis, Michael E., M.D., Medicine. Private Practice, Grand Rapids, Mich.

Enneking, William F., M.D., Orthopedic Surgery. Instructor, Department, Surgery, University of Chicago, Chicago, Ill.

Escabi, Luis Antonio, M.S., Protozoology. Chairman, Biology Department, Catholic University of Puerto Rico, San Juan, Puerto Rico.

Everett, Harold E., M.D., Internal Medicine. Private Practice, Northampton, Pa.

Everist, Bruce W., M.D., Pediatrics. Green Clinic, Ruston, La.

Ezrin, Calvin, M.D., Pathology. University of Toronto, Ont., Canada.

Farago, Peter Joseph, M.D., Clinical Research. Clinical Research Department, Abbot Laboratories, North Chicago, Ill.

Feldshuh, Alfred M., M.D., Biology. Private Practice, Kerhonkson, N.Y.

Ferguson, William W., Ph.D., Enteric Bacteria. Coordinating Bacteriologist, Division of Laboratories, Michigan Department of Health, Lansing, Mich.

Fernald, Willard Barker, M.D., Medicine. Chief Resident, Columbus Children's Hospital, Columbus, Ohio.

Fetscher, Charles A., Ph.D., Organic Chemistry. Director, Industrial Research, Nopco Chemical Company, Harrison, N.J.

Firminger, Harlan I., M.D., Experimental Pathology. Professor, Kansas University Medical Center, Kansas City, Kans.

Fishman, Jacob B., Ph.D., Physiology. Yale University, New Haven, Conn.

Fortgotson, James M., Jr., Ph.D., Geology. Research Engineer, Stanolind Research Center, Tulsa, Okla.

Forteza, Mario Enrique, M.D., Internal Medicine. Junior Physician Psychiatrist, Howard State Hospital for Mental Diseases, Howard, R.I.

Frederickson, Howard N., M.D., Medicine, Psychiatry. Private Practice, Baltimore, Md.

Freund, Jack, M.D., Clinical Pharmacology. Associate Director, Clinical Research, A.H. Robins Company, Richmond, Va.

Friedman, Nathan H., M.D., Biology. President, Staff, Bridgeport Hospital, Bridgeport, Conn.

Funke, Robert Edward, M.D., Internal Medicine. Assistant Medical Director, Prudential Insurance Company, Los Angeles, Calif.

Gartler, Stanley M., Ph.D., Physiological Genetics. Research Associate, Institute for the Study of Human Variation, Columbia University, New York, N.Y.

Gent, Donald H., M.D., Internal Medicine. Resident, Internal Medicine, Guthrie Clinic, Syre, Pa.

Gigante, Nicola, M.D., Medicine. Physician, Harper Hospital, Detroit, Mich.

Gilbert, Ivan, M.D., Medicine. Private Practice, Terre Haute, Ind.

Glauser, Stanley Charles, M.D., Biophysical Chemistry. Intern, Graduate Hospital, University of Pennsylvania, Philadelphia, Pa.

Glaviano, Vincent V., Ph.D., Physiology. New York Heart Association Fellow, Columbia University, New York, N.Y.

Goldberg, Harry, M.D., Cardiovascular Diseases. Assistant Professor, Medicine, Hahnemann Medical College, Philadelphia, Pa.

Goldberger, Emanuel, M.D., Medicine. Lecturer, Medicine, Columbia University, New York, N.Y.

Goldstein, Jacob D., M.D., Internal Medicine. Director, Department, Medicine, Brooklyn Jewish Hospital, Brooklyn, N.Y.

Goldthwaite, Joel C., M.D., Internal Medicine. Visiting Physician, Robert B. Brigham Hospital, Boston, Mass.

Goracci, Armando F., M.D., Surgery. Assistant, Surgery, Jefferson Medical College, Philadelphia, Pa.

Gordon, Maxwell, Ph.D., Organic Chemistry. Senior Scientist, Smith, Kline & French Laboratories, Philadelphia, Pa.

Gosline, Ernest, M.D., Neuropsychiatry. Supervising Psychiatrist, Rockland State Hospital, Orangeburg, N.Y.

Gottschall, Gertrude Y., Ph.D., Biochemistry. College of Physicians and Surgeons, Columbia University, New York, N.Y.

Graettinger, John Sells, M.D., Cardiology. Assistant Attending Physician, Presbyterian Hospital, Chicago, Ill.

Gray, Joseph E., M.S., Mycology, Bacteriology. Research Mycologist, Eaton Laboratories, Norwich, N.Y.

Green, Leonard Sanford, LL.B. Partner, L.S. Green Associates, New York, N.Y.

Green, Murray, D.D.S., Oral Surgery. Assistant Visiting Surgeon, Queens General Hospital, Jamaica, N.Y.

Griggs, Robert C., M.D., Hematology. Research Fellow, Hematology, City Hospital, Cleveland, Ohio.

Gumbreck, Laurence G., Ph.D., Endocrinology. Research Associate, Surgery, Washington University, St. Louis, Mo.

Haddox, Charles Hugh, Jr., Ph.D., Biochemical Genetics. Assistant Professor, Research, Louisiana State University, New Orleans, La.

Haight, Thomas Hulen, M.D., Internal Medicine. Assistant Professor, Medicine, University of Oklahoma, Oklahoma City, Okla.

Hamel, Helen, B.S., Medical Research. Junior Research Associate, Brookhaven National Laboratory, Upton, N.Y.

Handler, Alfred H., Ph.D., Heterologous Tissue Transplantation, Research Associate, Children's Cancer Research Foundation, Boston, Mass.

Hartman, John D., M.D., Biology. Assistant Professor, Anatomy, Temple University, Philadelphia, Pa.

Hedvall, Carl Erik Johan, M.D., Tuberculosis, Mycology. Head, University of Uppsala Lung Clinic, Uppsala, Sweden.

Hildick-Smith, Gavin, M.D., Pediatrics. Associate Medical Director, Squibb Institute for Medical Research, New Brunswick, N.J.

Hinko, Edward H., M.D., Psychiatry. Director, Cleveland Receiving Hospital, Cleveland, Ohio.

Hirschfeld, Gerhard R., M.D., Psychiatry. Associate Psychiatrist, St. Joseph's Hospital, Paterson, N.J.

Holland, Bernard, M.D., Internal Research. Principal Research Scientist, New York State Psychiatric Institute, New York, N.Y.

Hollis, Walter Jesse, M.D., Internal Medicine. Instructor, Louisiana State University, New Orleans, La.

Holloway, John E., M.D., Biophysics. Research Fellow, College of Medical Evangelists, Los Angeles, Calif.

Hsiung, Gueh-Djen, Ph.D., Medical Microbiology. Instructor, Yale University, New Haven, Conn.

Huf, Ernst G., Ph.D., General Physiology. Associate Professor, Physiology, Medical College of Virginia, Richmond, Va.

Hunter, Doris M., M.D., Psychiatry. Child Psychiatry, Western Psychiatric Institute, University of Pittsburgh, Pittsburgh, Pa.

Hunter, F. Edmund, Jr., Ph.D., Biochemistry. Associate Professor, Pharmacology, Washington University, St. Louis, Mo.

Jackson, Francis Charles, M.D., Surgical Diagnosis. Chief Surgeon, Veterans Administration Hospital, Pittsburgh, Pa.

Jacobson, Jerry H., M.D., Electrophysiology of the Eye. Director, Department, Electrophysiology, New York Eye and Ear Infirmary, New York, N.Y.

James, G. Watson, III, M.D., Internal Medicine. Associate Professor, Medicine, Medical College of Virginia, Richmond, Va.

Jamison, William L., M.D., Cardiovascular. Cardiovascular and Thoracic Surgeon, Charleston, W. Va.

Jana, Joseph T., Jr., M.D., Biology. Private Practice, Miami, Fla.
Jarvik, Murray, M.D., Psychology. Assistant Professor, Pharmacology, Albert Einstein College of Medicine, New York, N.Y.
Jawetz, Ilse K., M.D., Psychiatry. Assistant, Psychiatry, Mt. Zion Hospital, San Francisco, Calif.
Jenkins, Rose D., M.D., Psychiatry. Resident, Neuropsychiatry, Freedmen's Hospital, Washington, D.C.
Jensen, David, Ph.D., Physiology. Fellow, Los Angeles County Heart Association, Research Service, Veterans Administration Center, Los Angeles, Calif.
Jolley, John W., M.D., Biology. Private Practice, Delray Beach, Fla.
Jonas, Murray, M.D., Psychiatry. Private Practice, New York, N.Y.
Jones, Chase Breese, Ph.D., Biochemistry. Director, Biochemical Research, Gillette Safety Razor Company, Boston, Mass.
Jones, Henry, Jr., Phar. D., X Ray. Chief, X-Ray Technical Service, Maricopa County General Hospital, Phoenix, Ariz.
Jordan, Alexander Boyd, M.D., Internal Medicine. Assistant Resident, Medical Services, Montefiore Hospital, New York, N.Y.
Judy, Harriet E., M.D., Endocrinology. Private Practice, Spokane, Wash.
Jumel, Mignon W., M.D., Medicine. Louisiana State University, New Orleans, La.
Jung, Haw Chan, M.D., Pathology. Associate Pathologist, St. Luke's Hospital, San Francisco, Calif.
Kahana, Lawrence, M.D., Medicine. Medical Officer, U.S. Public Health Service, Memphis, Tenn.
Kelly, Luther W., Jr., M.D., Endocrinology. Associate Physician, Nalle Clinic, Charlotte, N.C.
King, John McKain, D.V.M., Pathology. Assistant, New York State Veterinary College, Ithaca, N.Y.
Kinosita, Riojun, Ph.D., Biology. Chairman, Department, Experimental Pathology, City of Hope Medical Center, Duarte, Calif.
Kinross-Wright, Vernon, M.D., Neuropsychiatry. Associate Professor, Baylor University, Houston, Texas.
Koch, Richard, M.D., Infectious Disease. Outpatient Director, Los Angeles Children's Hospital, Los Angeles, Calif.
Koff, Gilbert Yale, M.S., Pharmacology. Assistant, Jefferson Medical College, Philadelphia, Pa.
Kolb, Felix O., M.D., Endocrine Diseases. Assistant Clinical Professor, University of California School of Medicine, San Francisco, Calif.
Kolter, Samuel, M.D., Biochemistry. Supervising Psychiatrist, Middletown State Hospital, Middletown, N.Y.
Komfeld, Peter, M.D., Cardiovascular Diseases, Physiology. Chief Resident, Department, Medicine, Mt. Sinai Hospital, New York, N.Y.
Kraft, Irvin A., M.D., Psychiatry. Research Fellow, Child Psychiatry, Tulane Medical School, New Orleans, La.
Lack, Charles, B.S., Pathology. Pathologist, Royal National Orthopaedic Hospital, Stanmore, Middlesex, England.
Larson, Carl A., Lic. Med., Human Genetics. First Assistant, Institute of Genetics, University of Lund, Lund, Sweden.
Laskin, Allen I., Ph.D., Industrial Microbiology. Research Associate, Squibb Institute for Medical Research, New Brunswick, N.J.
Ledbetter, John Paul, M.D., Medicine. Physician, Jonesboro, Ark.
Lederman, Leon M., Ph.D., Physics. Associate Professor, Physics, Columbia University, New York, N.Y.
Levin, Joseph, M.D., Medicine. Attending Physician-Chief, Division of Medicine, Harrison Martland Medical Center, Newark, N.J.
Levitt, Morton, Ph.D., Psychology. Assistant Professor, Wayne University, Detroit, Mich.
Lightstone, Clifford, D.O., Psychology. Private Practice, Dearborn, Mich.
Lipschutz, Arthur, M.D., Pediatrics. Assistant Professor, Hahnemann Medical College, Philadelphia, Pa.
Little, George N., Mycology. Junior Bacteriologist, Division of Laboratories and Research, New York State Health Department, Albany, N.Y.
Little, Joseph A., M.D., Pediatric Cardiology. Associate Professor, Pediatrics, University of Louisville, Louisville, Ky.

Livingston, Robert T., Industrial Management, Engineering. Professor, Industrial and Management Engineering, Columbia University, New York, N.Y.

Lucas, Fred V., M.D., Pathology. Associate Professor, Pathology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Lyons, Harold A., M.D., Cardiopulmonary Diseases. Associate Professor, Medicine, Director, Cardiopulmonary Laboratory, State University of New York, Brooklyn, N.Y.

McGrath, Mary Catherine, B.S., Bacteriology. Bacteriologist, Mt. Carmel Mercy Hospital, Detroit, Mich.

Maiese, Michael R., M.S., Medical Sciences. Research Director, Ives-Cameron Company, Division American Homes Products Corp., Philadelphia, Pa.

Maluf, Noble S. R., M.D., Physiological Aspects of Urology. Staff Urologist, Kaiser Foundation Hospital, Los Angeles, Calif.

Marano, R. J., M.S., Biology. Associate Professor, Chairman, Biological Sciences, Fordham University, New York, N.Y.

March, Cyril, M.D., Pharmacology. Research Fellow, Skin and Cancer Unit, Bellevue Medical Center, New York, N.Y.

Melby, James C., M.D., Endocrinology. Resident, Medicine, University of Minnesota, Minneapolis, Minn.

Mellen, Hyman Samuel, M.D., Cardiovascular Disease. Attending Cardiologist, Grace-Sinai Hospital, Detroit, Mich.

Meritt, J. E., Jr., M.D., Medicine. Private Practice, Raleigh, N.C.

Mitchell, Leonard, Ph.D., Medicinal Chemistry. Director, Research, Frank W. Horner Ltd., Montreal, P.Q., Canada.

Muelling, Rudolph J., Jr., M.D., Pathology. Assistant Professor, Pathology, Louisiana State University, New Orleans, La.

Myles, Martin Burt, M.D., Pulmonary Diseases. Assistant Resident, Internal Medicine, TV Division, Bellevue Hospital, New York, N.Y.

Nannarello, Joseph, M.D., Psychiatry. Private Practice, Greenville, S.C.

Nedelkoff, J.M., M.D., Medicine. Private Practice, Salineville, Ohio.

Neighbor, E.G., M.D., Biology. Private Practice, Kansas City, Kans.

Nelson, Richard M., M.D., Neuropsychiatry. Cleveland Clinic Foundation, Cleveland, Ohio.

Neu, Harold Nicholas, M.D., Internal Medicine. Professor, Medicine, Creighton University, Omaha, Nebr.

Neustadt, David Harold, M.D., Rheumatic Disease. Private Practice, Louisville, Ky.

Newberne, James W., D.V.M., Experimental Pathology. Pathologist, Research Division, Pitman-Moore Company, Indianapolis, Ind.

Noble, Nancy Lee, Ph.D., Biochemistry. Director, Biochemistry, Howard Hughes Medical Institute, Miami, Fla.

Norris, Myron, B.S., Analog Computers, Electrical Engineer. Avion Division A.C.F., Paramus, N.J.

Nygren, Axel, Ph.D., Genetics. Associate Professor, Genetics, Institute of Genetics, University of Uppsala, Uppsala, Sweden.

Okita, George T., Ph.D., Cancer Research. Research Associate, Argonne Cancer Research Hospital, Chicago, Ill.

Olash, F. Albert, M.D., Medicine. Instructor, Medicine, University of Louisville, Louisville, Ky.

Oliner, Leo, M.D., Endocrinology. Physician in Charge, Radioisotope Laboratory, Michael Reese Hospital, Chicago, Ill.

Oloffson, Olaf, Medicine. Vice President, Copy Director, Cortez F. Enloe, Inc., New York, N.Y.

Oppenheim, Elliot, M.D., Biology. Private Practice, New York, N.Y.

Oppenheimer, Bertram J., M.D., Biology. Private Practice, Yonkers, N.Y.

Oppenheimer, Morton Joseph, M.D., Physiology. Professor, Physiology, Temple University, Philadelphia, Pa.

Page, W. Randolph, M.D., Neurosurgery. Private Practice, New Orleans, La.

Pallotta, Arthur J., Ph.D., Pharmacology. Pharmacologist, Falls Church, Va.

Palma, Louis Stephen D., M.D., Surgery. Surgical Resident, Massachusetts Memorial Hospital, Boston, Mass.

Peck, David Rex, Electroencephalography. Lenox Hill Hospital, New York, N.Y.

Petersen, Donald Francis, Ph.D., Pharmacology. Instructor, Department, Pharmacology, Research Associate, U.S. Air Force Radiation Laboratory, University of Chicago, Chicago, Ill.

Randall, Robert Godfrey, M.D., Plastic Surgery. Assistant Visiting Plastic Surgeon, Kings County Hospital, Brooklyn, N.Y.

Rasko, Eugene, M.D., Cardiology. Assistant Visiting Physician, Medical Department, Sydenham Hospital, New York, N.Y.

Reichsman, Franz, M.D., Internal Medicine. Assistant Professor, Medicine, Strong Memorial Hospital, Rochester, N.Y.

Rippy, Wilson C., Jr., M.D., Psychiatry. Resident, Neurology, Roanoke, Va.

Romano, Dominick V., B.S., Pharmacology. Research Assistant, Department, Pharmacology, U.S. Vitamin Corporation, Yonkers, N.Y.

Rothman, Gertrud R., M.D., Biology. Private Practice, Psychology, Los Angeles, Calif.

Rousell, P.G., M.S., Insect Physiology. Assistant Professor, Department, Biology, St. Francis Xavier University, Nova Scotia, Canada.

Sandusky, William R., M.D., Biology. University of Virginia Hospital, Charlottesville, Va.

Schiff, Irwin, M.D., Biology. Private Practice, Brooklyn, N.Y.

Schweig, Karl, M.D., Pediatrics. Visiting Pediatrician, Montefiore Hospital, New York, N.Y.

Sebrell, William Henry, Jr., M.D., Nutrition. Research Consultant, American Cancer Society, New York, N.Y.

Segerman, Ephraim, M.S., X-ray Crystallography. Physics Consultant, Department, Pathology, State University Medical School, Brooklyn, N.Y.

Sevy, Roger W., M.D., Pharmacology. Assistant Professor, Pharmacology, Temple University, Philadelphia, Pa.

Shands, Harley C., M.D., Psychiatry. University of North Carolina, Chapel Hill, N.C.

Shuman, H. Herman, M.D., Epidemiology. Pediatrist, Springfield Hospital, Springfield, Mass.

Siim, J. Chr., M.D., Infectious Diseases. Chief, Toxoplasma Laboratories, State Serum Institute, Copenhagen, Denmark.

Smith, Richard T., M.D., Experimental Pathology. Senior Fellow, Arthritis and Rheumatism Foundation, University of Minnesota, Heart Hospital, Minneapolis, Minn.

Sullivan John F., M.D., Neurology. Chief, Neurology, New England Center Hospital, Boston, Mass.

Szappanyos, B. J., M.D., Medicine. Wayne County General Hospital, Eloise, Mich.

Szirmai, John A., M.D., Connective Tissues. Research Associate, Research Laboratories, University of Leiden, Leiden, Holland.

Tacket, Hall Sanford, M.D., Biology. Assistant Professor, Medicine, University of Tennessee, Memphis, Tenn.

Taylor, Graham C., M.D., Psychiatry. Assistant Director, Allan Memorial Institute, Montreal, P.Q., Canada.

Taylor, Richard M., Ph.D., Pharmacology. Pharmacology Research, White Laboratories, Kenilworth, N.J.

Tedeschi, Ralph E., Ph.D., Neurology. Senior Pharmacologist, Smith, Kline & French Laboratories, Philadelphia, Pa.

Teodoru, Constantin V., M.D., Physiology. Research Associate, Mt. Sinai Hospital, New York, N.Y.

Timmerman, Raymond Joseph, M.D., Gastroenterology. Research Fellow, Department, Internal Medicine, Harvard University, Boston, Mass.

Townshend, Wilfred H., Jr., M.D., Internal Medicine. Associate, Medicine, Maryland Medical School, Baltimore, Md.

Turner, Richard H., D.O., Medicine. Resident Physician, Detroit Osteopathic Hospital, Detroit, Mich.

Turner, W. J., M.D., Psychiatry. Private Practice, Huntington, N.Y.

Turvey, S. E. C., M.D., Biology. Private Practice, Vancouver, B.C., Canada.

Ullman, Grace E., B.A., Bacteriology. Graduate Student, Jefferson Medical College, Philadelphia, Pa.

Unger, Pat Benjamin, M.D., Anthropology. U.S. Navy, San Francisco, Calif.
Unruh, H. A., M.D., Experimental Glomerulonephritis. General Medicine, C. S. Williams Clinic, Rossland, B.C., Canada.
VandenBerg, Henry J., Jr., Cancer. Private Practice, Detroit, Mich.
Van Noord, Gelmera, M.D., Psychiatry. Assistant Superintendent, Pine Rest Sanitorium, Grand Rapids, Mich.
Vaughan, John H., M.D., Medicine. Assistant Professor, Medical College of Virginia, Richmond, Va.
Vishniac, Roman, Ph.D., Protozoa. New York, N.Y.
Waltcher, Irving, Ph.D., Polymer Chemistry. Assistant Professor, College of the City of New York, New York, N.Y.
Washko, F.V., D.V.M., Veterinary Medicine. Veterinary Pathologist, Merck & Co., Inc., Rahway, N.J.
Weisskopf, Alex, M.D., Physiology. Private Practice, San Mateo, Calif.
Witteborg, Lothar P., M.A., Anthropology. Chief, Art and Exhibition Department, American Museum of Natural History, New York, N.Y.
Wolfe, John J., M.D., Medicine. Instructor, Department, Surgery, University of Louisville, Louisville, Ky.
Wood, Henry Nelson, Ph.D., Biochemistry. Assistant, Rockefeller Institute for Medical Research, New York, N.Y.
Yard, Allan S., Ph.D., Pharmacology. Assistant Professor, Rutgers University, Newark, N.J.
Zatuchni, Jacob, M.D., Medicine. Consultant, Internal Medicine, Central Diagnostic Chest Clinic, Philadelphia, Pa.
Zeman, Wolfgang, M.D., Neurology. Assistant Professor, Pathology, University of Pittsburgh, Pittsburgh, Pa.
Zweng, Harold Christian, M.D., Ophthalmology. Staff Member, Palo Alto Clinic, Palo Alto, Calif.

STUDENT MEMBERSHIP

Glickman, David, Pharmacology. Student, Columbia University College of Pharmacy, New York, N.Y.
Green, Richard S., M.D., Psychiatry. Payne Whitney Institute, Brooklyn, N.Y.
Nussbaum, Morton. Student, Chiropractic Institute of New York, New York, N.Y.